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#Microbio24

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POSTER ABSTRACT BOOK

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BLOCK A

Session : Environmental and Applied Microbiology Forum

A001

Impact of Physico-Chemical parameters on the growth of actinomycetes isolated from the coastal areas of Arabian sea

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Abstract

The marine actinomycetes are a rich source for novel biomolecules. Especially the exotic tropical marine habitat of the Kerala coastal region favours the actinomycete diversity. The present study focuses on the isolation, purification and morphological characterization of marine actinomycetes. A total of 280 morphologically distinct actinomycetes were isolated from marine soil and sediments of 10 different isolation sites along the coastal region of Thiruvananthapuram district Kerala, India. The physicochemical analysis of the soil samples collected from different stations was also done. Even though the soil and sediment samples were collected from geographically nearby places, the physicochemical parameters showed a significant variation. This may be one of the factors which may trigger the actinomycete diversity in these regions.

A007

Phaeoviruses present in cultured and natural kelp species, *Saccharina latissima* and *Laminaria hyperborea* (Phaeophyceae, Laminariales), in Norway.

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Abstract

Kelps, brown algae (Phaeophyceae) from the order Laminariales, dominate in shallow subtidal rocky habitats in most temperate coastal areas around the world. Kelp forests are structurally complex and highly productive habitats that enhance local biodiversity and support food webs through secondary production. Kelp forests are under pressure due to herbivore outbreaks, eutrophication, ocean warming and/or other climate induced changes. It has been estimated that about 38% of the world's kelp forests have disappeared over the past five decades due to these changes; however, epidemiological knowledge of seaweed pathogens in European species is very poor.

Phaeoviruses (Phycodnaviridae) are large icosahedral viruses in the phylum Nucleocytoviricota with dsDNA genomes ranging from 160 to 560kb infecting multicellular brown algae. Phaeoviruses were known to infect algae from the Ectocarpales, but their host-range is broader than expected, also infecting algae from the Laminariales order. Despite phaeoviral infections have been reported globally, Norwegian kelp species had not been screened before. Therefore, we carried a molecular analysis of cultured and wild samples of two economically important kelp species in Norway (*Saccharina latissima* and *Laminaria hyperborea*), which showed the highly prevalence of these viruses along the Norwegian coast. Moreover, we found up to three different viral sequences in the same algal individual, one of which does not belong to the Phaeovirus genus and has never been reported before.

A008

Risk assessment of quaternary ammonium compounds in hygiene-relevant microorganisms: cross-resistance, susceptibility, and effects on negative controls

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Abstract

Risk assessment of biocide exposure frequently relies on studies that involve exposure of pure bacterial cultures to aqueous biocide solutions. We passaged 10 microbial species (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* (DSM 682 and K12), *Enterococcus hirae*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Bacillus subtilis*, *Candida albicans* and *Salmonella enterica*) 10 times on concentration gradients of 5 quaternary ammonium compounds and then 10 times in biocide-free media. Controls were passaged 20 times in biocide-free media. Isolates were tested for susceptibility to 5 cationic biocides and 10 antibiotics. Changes in biocide susceptibility were similar between exposed (10/10 organisms, fold change 0.7-16) and negative controls (10/10 organisms, fold change 0.3-2.7). All test bacteria (10/10) exhibited changes in biocide susceptibility to 5 biocides (fold changes between 0.3 and 16, mean = 2.5) and both increased (9/10) and decreased (9/10) antibiotic susceptibilities (zone size changes compared to parental strain between 18mm and -34mm). *E. coli* K12 had increased susceptibility to all ten antibiotics when exposed to ADBAC C12 and C14. *S. aureus* exhibited the greatest number of decreases (172/180) and the largest decreases in inhibition zone diameters. DDAC and DTAC decreased antibiotic susceptibility beyond EUCAST clinical breakpoints for imipenem, piperacillin, ticarcillin, ciprofloxacin and kanamycin in *E. coli K12, K. pneumoniae, S. aureus* and *E. hirae*, although we observed similar changes in controls (4/4 organisms). We have demonstrated that decreased antimicrobial susceptibility in biocide-exposed bacteria may also occur following passage in the absence of biocide. Antibiotic susceptibility data were similar between test organisms and controls.

A011

Biological and synthetic surfactants increase class I integron prevalence in ex-situ biofilms

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Abstract

The role of biocides in the spread of antimicrobial resistance (AMR) has been addressed but only few studies focus on the impact of surfactants on microbial diversity and AMR, although they are common constituents of cleaners, disinfectants and personal care products and thus are released into the environment in large quantities. In this study, we used an ex situ biofilm model to examine the development of four biofilms exposed to surfactants and analyzed the biofilms for their prevalence of class I integrons (intI1). We furthermore determined the bacterial community composition by high-resolution melt analysis and 16S ribosomal DNA sequencing. Depending on the initial intrinsic prevalence of intI1 in the respective ex situ biofilm, Benzalkonium Chloride, Alkylbenzene Sulfonate and Cocamidopropyl Betaine increased intI1 prevalence by an average of up to six fold. For surfactants, such as Fatty Alcohol Ethoxylate and the biosurfactants Sophorolipide and Rhamnolipide, the mean increase in intI1 prevalence did not exceed two fold. Across all surfactants the increase of intI1 was accompanied by a shift in bacterial community composition. Here especially Benzalkonium Chloride, Cocamidopropyl Betaine and Alkylbenzene Sulfonate changed the communities, while Fatty Alcohol Ethoxylate, Sophorolipide and Rhamnolipide had a lower effect on the bacterial biofilm composition.

A013

Manipulation of the yeast metabolic pathway to boost mycosporine-like amino acid production in *S. cerevisiae*

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Abstract

Mycosporine-like amino acids (MAAs) are a class of strong UV-absorbing compounds produced by cyanobacteria, algae and corals and are promising candidates as natural sunscreen components. MAAs are produced from either the PPP metabolite sedoheptulose-7-phosphate, or the shikimate pathway intermediate 3-dehydroquinate, by the sequential action of 4 enzymes. The low yields from natural sources, coupled with difficulties associated with culturing its native producers, have resulted in efforts to use a synthetic biology approach to produce MAAs in tractable hosts. We produced the MAA shinorine (mycosporine-glycine-serine) in *Saccharomyces cerevisiae* by expressing the four enzymes required for its biosynthesis. We show that MAAs are produced from sedoheptulose-7-phosphate (S7P), and not from 3-dehydroquinate (3DHQ). Deletion of TAL1, which splits S7P into erythrose-4-phosphate (E4P) and glyceraldehyde-3-phosphate (G3P), boosts S7P / shinorine production. Inhibiting glycolytic flux also enhances S7P/ shinorine production. Deletion of TAL1 and reducing glycolytic flux had an additive effect on shinorine production, suggesting that the modifications work via different pathways. Our results on how reducing glycolytic flux boosts S7P production in *S. cerevisiae* will be presented in the poster.

A015

Isolation and Identification of Antibiotic-Producing Bacteria in New Jersey Soil Samples

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Abstract

Antimicrobial resistance is a growing concern that poses a threat to food security, societal development, and global health. A new generation of effective antibiotics is urgently needed to combat the emergence of novel and stronger pathogenic microbe variants. The Tiny Earth project is an attempt to contribute to this initiative through the collection, isolation, and identification of antibiotic-producing bacteria from different soil samples. The soil samples were obtained from Somerset County, New Jersey, and a serial dilution method was used to plate and isolate distinct bacteria specimens. From the obtained sample, bacteria #13 displayed antibiotic properties against *E. Coli*, *Staphylococcus epidermidis*, and others through the observed halo formed after patch plating. After an array of metabolic tests such as MR/VP, the bacteria of interest was identified to be a gram-negative facultative anaerobe that can ferment both glucose and lactose and produce H_2S. Further identification was facilitated with the use of a dichotomous key and PCR sequencing. Based on a dichotomous key, the antibiotic-producing bacteria of interest was identified as Citrobacter. However, the PCR testing and BLAST database evidenced that the unknown might be a variation of Pseudomonas.

Further research would focus on the extraction of chemicals from bacteria with possible antibiotic activity. The program would also involve the introduction of novel antibiotics to the market to alleviate a dwindling effective antibiotic supply.

A016

Marine microbial communities of Menai Strait (Irish Sea, UK) capable of degrading long-chain *n*-alkanes.

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Abstract

Degradation of long-chain *n*-alkanes (C_{18}-C_{30}) in the marine environment is generally less efficient due to their low water solubility and low bioavailability to microorganisms. The study of marine microbial communities capable of degrading long-chain alkanes will help to find an optimal bioremediation strategy for biodegradation of these substrates.

The aim of this work was to study the composition, diversity, and changes in community structure of hydrocarbon degrading marine bacteria growing on C_{18} as sole carbon in the presence of nitrogen source and high salinity.

Enrichments were carried out on paraffin C_{18} using surface seawater samples from Menai Strait (Irish Sea, UK) supplemented with ammonium chloride and sodium chloride. Incubation of the enriched samples was carried out at room temperature for two months. The composition and diversity of bacterial communities in the enriched media were analysed by 16S rRNA gene sequencing on the MiSeq Illumina platform.

Analysis of sequencing data showed that C_{18} alkanes, nitrogen source and increased salinity led to an increase in *gamma*- and *alphaproteobacteria* and the isolation of different types of bacterial communities. Enrichment of seawater on paraffin alone showed dominance of the genera *Oleibacter*, *Alteromonas* and *Maricaulis*, while addition of ammonium chloride and sodium chloride promoted the genera *Alcanivorax*, *Roseivirga* and *Owenweeksia*. Thus, this study showed that changes in the structure of hydrocarbon-degrading communities grown on C_{18} paraffin wax were induced by the addition of nitrogen source and increased salinity.

A017

Occurrence, resistance, and biofilm profile of selected gram-negative bacteria isolated from poultry sources in Akoko regions of Ondo State, Nigeria

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Abstract

The indiscriminate use and overuse of various antibiotics have caused the rapid emergence of antibiotic-resistant bacteria (ARB) in poultry products and the surrounding environment, leading to global public health issues. This study determined the prevalence of multidrug-resistant (MDR) and gram-negative bacteria (GNB) found in the environment of poultry farms in Akoko North local government area of Ondo State.

Poultry products and poultry environment samples were collected from nine (9) poultry farms and were cultured for GNB using MacConkey and Xyline Lysine Deoxycholate (XLD) media. The GNB were identified using the Microbact® 24E Identification Kit and also subjected to an antimicrobial susceptibility test (AST).

The identified GNB isolates were Salmonella spp., Aeromonas hydrophilia, Enterobacter spp., Klebsiella pneumoniae, Proteus spp., Pseudomonas aeruginosa, Escherichia coli, and Shigella spp. AST results showed that 20% of the GNB isolates were resistant to ciprofloxacin, 10% were resistant to tetracycline, and the other isolates were susceptible to other antibiotics. Seven of the isolates two strains of Pseudomonas aeruginosa, Enterobacter agglomerans, Escherichia coli, and one strain of Salmonella typhi were resistant to more than one antibiotic, with MAR indices ranging from 0.50 to 0.67. It was seen that the isolates of Pseudomonas aeruginosa and Escherichia coli formed strong biofilms (optical density: 0.76-0.92), while the isolates of Salmonella typhi and Enterobacter agglomerans formed moderate-to-weak biofilms (optical density: 0.32-0.60).

The presence of biofilm-forming GNB strains and the frequency of ARB and MDR bacteria in the poultry products pose a potential public health threat to workers and the entire neighboring community.

A019

Investigating the antibacterial potential of selenium nanoparticles with varied coatings and their bacteria-induced allotropic transformation.

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Abstract

The use of metal nanoparticles (NPs) as antimicrobial agents has become a promising alternative to the problem of antibiotic-resistant bacteria. In this sense, selenium nanoparticles (SeNPs) gaining more attention for their effective biocide properties against bacteria. This study aims to investigate the antibacterial activity of chemically synthesized SeNPs with different surface coatings (BSA-coated, chitosan-coated, and undefined coating) on bacterial models such as *Stenotrophomonas bentonitica* (gram-negative) and *Lysinibacillus sphaericus* (gram-positive). The tested NPs had similar properties, including shape (spheres), structure (amorphous), and size (50-90 nm), but differed in their surface charge. We have found that cell growth and viability of both bacteria were negatively affected in the presence of the NPs as indicated by microcalorimetry and flow cytometry. Specifically, undefined coating SeNPs displayed the highest percentage values of dead cells for both bacteria (85-91%). An increase in reactive oxygen species (ROS) production was also detected. Chitosan-coated and undefined SeNPs caused the highest amount of ROS (299.7 and 289% over untreated controls) for *S. bentonitica* and *L. sphaericus*, respectively. Based on DNA degradation levels, undefined-SeNPs were found to be the most hazardous, causing nearly 80% DNA degradation. Finally, electron microscopy revealed the ability of the cells to transform the different SeNP types (amorphous) to crystalline SeNPs (trigonal/monoclinical Se). The results obtained herein demonstrate the promising potential of SeNPs for their use in medicine as anti-microbial agents and we propose *S. bentonitica* and *L. sphaericus* as candidates for new bioremediation strategies and nanoparticle synthesis with potential applications in many fields.

A020

Surviving Beyond the Gut: Investigating Aerotolerance Evolution in *Campylobacter jejuni*

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Abstract

Campylobacter jejuni (*C. jejuni*) is the most common cause of bacterial diarrhoeal disease globally. *C. jejuni* colonises the gastrointestinal tract and isolated samples commonly show signatures of adaptation, enabling survival in this low oxygen environment. However, isolates have also been identified from a diverse range of sources, including water, poultry production lines, and dairy products, suggesting the potential to also tolerate higher oxygen concentrations, termed aerotolerance. Aerotolerance facilitates transmission, prolonging environmental survival and reducing the need for host-to-host contact. This contradicts *C. jejuni's* fastidious nature and raises questions of how differences in individual isolates influence extra-intestinal survival and transmission. We challenged the *C. jejuni* lab strain NTC11168 and seven environmental isolates, using serial passage and exposure to atmospheric conditions to evolve aerotolerance. We determined change in aerotolerance by performing serial dilutions of the isolates throughout, spotting known volumes onto agar plates, and subsequently counted colonies to calculate colony-forming units (CFUs) per milliliter. The evolved lines were sequenced and compared to the original ancestral strains and controls, facilitating the identification of candidate genes potentially involved in stress and aerotolerance. Our study aims to deconstruct the genetic basis of adaptability of *C. jejuni* to atmospheric oxygen levels that facilitate transmission. Further investigation and research of identified genes to understand their role in survival will inform targeted interventions and control measures to reduce *C.jejuni* infection.

A021

Characterisation of the microbial population within the *Agaricus bisporus* casing layer and how it is impacted by crop cycle progression and biological treatment application

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Abstract

Agaricus bisporus (white button mushroom) is the most cultivated mushroom species in the UK and Ireland. It is recognised that the mushroom industry will need to establish novel integrated pest management strategies involving the use of biological control agents (BCAs). The aim of this work was to establish if addition of BCAs (*Bacillus velezensis* QST 713 & *B. velezensis* Kos) significantly impacted the casing population dynamics of an *A. bisporus* crop. 16S and ITS amplicon sequencing was performed at several timepoints over the course of the crop cycle. At day 0, four bacterial phyla dominated the casing layer; (*Firmicutes* (25.93%,+/- 5.38 SD), *Proteobacteria* (24%,+/- 2.86 SD), *Bacteroidota* (16.88%,+/- 4.40 SD) and *Actinobacteriota* (14.62%,+/- 2.34 SD)). As the crop cycle progressed, *Proteobacteria* became the dominant phylum accounting for 54.41% (+/- 2.93 SD) of all ASVs by T7, while *Firmicutes* decreased to 4.37% (+/-1.48 SD). When BCA treated casing samples were compared to the control, at all timepoints, there was no significant differences observed for alpha diversity measures, indicating no differences in species richness or evenness between treatment plots. In terms of beta diversity measures, there were some significant differences between treatments however inspection of sample pairwise comparisons did not reveal any significant differences between individual groups. For fungal diversity, the *Basidiomycota* phylum and *Agaricus* genus accounted for the majority of detected ASVs, 99.85% (+/- 0.16 SD) and 99.81% (+/- 0.16 SD) respectively. Overall, the addition of BCA to the casing layer did not significantly alter the bacterial and fungal populations during *A. bisporus* crop cycle.

A022

Environmentally Relevant Concentrations of Erythromycin and Sulfamonomethoxine Antibiotics Alter Fresh Water Sediment Bacterial Communities

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Abstract

Antibiotic resistance (ABR) is a public health threat. Antibiotic use in human/animal care has resulted in increased antibiotic pollution. In aquatic environments, antibiotics have been detected at concentrations ranging from ng/L- µg/L. At sub-inhibitory concentrations, antibiotics can induce mutagenesis and horizontal gene transfer in bacteria, resulting in the acquisition of ABR. Current levels of antibiotic pollution in the environment could be driving the persistence and selection of ABR and altering bacterial community composition (BCc).

We assessed the effect of erythromycin and sulfamonomethoxine at concentrations of 0.1 µg/L and 10 µg/L on a freshwater sediment bacterial community (BC) over 60 days. Changes in BCc in response to antibiotic exposure were assessed via metataxonomic sequencing of the V4 hypervariable region of bacterial 16S rDNA. Genes involved in erythromycin (*oleC*, *ereA*), sulfamonomethoxine (*sul2*), and multidrug (*mexB, mdtA*) resistance were quantified by real-time PCR to evaluate changes in abundance in response to antibiotic amendment.

We observed that exposing BC to low levels of erythromycin and sulfamonomethoxine increases diversity and alters the composition of the BC. The diversity increase was explained by moderately abundant taxa that can potentially degrade antibiotics or serve as hosts to ABR determinants. We also observed a significantly higher mean abundance of the multidrug efflux pump gene *mdtA* relative to 16S rDNA in erythromycin and sulfamonomethoxine-amended and unamended compared to all the genes quantified.

These results indicate that current levels of antibiotic pollution can alter bacterial community structure and promote the persistence of ABR genes in the environment.

A023

Discovery of novel carboxylesterases from environmental metagenomes and microbial genomes for recycling of synthetic polyesters

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Abstract

Enzymatic depolymerization of synthetic polymers represents an attractive approach for developing efficient plastics recycling technologies and reducing environmental plastic pollution. Although several polyester-degrading enzymes have been identified and characterised to date, the efficient enzymatic depolymerisation of synthetic polyesters still presents several challenges including a limited repertoire of available enzymes with highly similar sequences, which do not reflect the potential biochemical diversity of environmental metagenomes and microbial genomes. In addition, the thermostability and activity of known polyesterases are unsatisfactory for plastics recycling under industrial conditions. Therefore, the identification and characterisation of new classes of polyester-degrading enzymes with high activity and stability are urgently needed for developing new biocatalysts for plastics recycling and upcycling. Recently, the discovery of carboxylesterase IS12 from the hydrothermal vent of Italy's Ischia Island showed remarkable thermostability and efficacy against various synthetic polyesters (PET, PCL, PLA). To identify novel highly active and stable enzymes for applications in polyester recycling, we are screening purified uncharacterised carboxylesterases from environmental metagenomes and microbial genomes for polyesterase activity against several polyesters (PET, PLA, PCL) using indicator plates and HPLC. Screens revealed 26 carboxylesterases from different metagenomes and microbial genomes have polyesterase activities. Polyesterase activity and stability of the four most active enzymes were further characterised, and the thermostability of one natural enzyme was greatly improved using ancestral sequence reconstruction. Thus, this study identified novel polyesterases with high stability and activity against a broad range of synthetic polyesters for potential applications in plastics recycling and sustainable environmental practices.

A024

Bifidobacterium longum ITT13 – A probiotic candidate.

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Abstract

Probiotics are considered to be live strains of selected microorganisms that have been shown to confer health benefits on the host when administered in adequate amounts. The introduction of probiotics into the human diet is thought to favourably modulate the intestinal microbiota with benefits for the host such as alleviation of gastrointestinal diseases (e.g. inflammatory bowel disease and diarrhoeal disease) and in other conditions where dysbiosis contributes to a variety of systemic diseases (e.g. type 1 diabetes).

Bifidobacterium longum is a known inhabitant of the human gut. In this study, *Bifidobacterium longum* ITT13, originally isolated from a neonate faecal sample, was assessed for its suitability as a probiotic strain. In order to be designated as a probiotic, candidate strains must meet certain criteria in the areas of safety, functionality and technological usability.

In this research, the effects of selected prebiotics and co-fermentation with a number of lactic acid bacteria on the growth and antimicrobial activity of *B. longum* ITT13 were evaluated. Furthermore, the biofilm-forming capacity of *B. longum* ITT13 and the anti-biofilm forming properties of the organism's fermentate were assessed. The antibiotic resistance profile of the organism was also determined.

Results to date indicate the potential for consideration of *B. longum* ITT13 as a probiotic.

Further studies aim to sequence the organism's genome and its ability to work synergistically with antibiotics against indicator gut pathogens.

A026

Deciphering the roles of *Escherichia coli* encoded Lon protease in the metabolism of 2,4-Dinitrophenol

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Abstract

In prokaryotes, the majority of energy-dependent protein degradation is controlled by two ATP-dependent proteases: Lon and ClpP. This study investigates the roles of *Escherichia coli* (*E. coli*) encoded Lon protease in the metabolism of 2,4-Dinitrophenol (2,4-DNP), a toxic industrial compound. Our laboratory has been studying the roles of Lon protease and downstream regulators in the induction of phenotypic antibiotic resistance or resistance observed in the presence of compounds such as sodium salicylate, acetaminophen, glyphosate, etc. A previous study in the lab explored the potential of uncouplers, e.g. 2,4-DNP and CCCP, in the induction of phenotypic antibiotic resistance. During the study, an observation was made that a strain lacking Lon protease (Δ*lon*) resulted in an enhanced production of a reddish-brown product compared to the conversion by the wild-type (WT) strain. This study aims to characterise the compound observed in the media with WT and Δ*lon* strains, understand the mechanisms of 2,4-DNP conversion and decipher the roles of Lon protease in the conversion of 2,4-DNP. UV-visible and LC-MS analyses revealed differences in the conversion products between the wild-type and Δ*lon* strains. Further exploration implicated nitro reductases, focusing on their regulation by MarA, a transcription factor. Growth studies with different mutants and trans-complemented strains indicated MarA-dependent conversion. The enhanced conversion capabilities of the Δ*lon* strain suggest its potential application in bioremediation efforts for nitroaromatic decontamination for reduction processes. This study contributes to understanding the biological treatment of nitroaromatics, offering insights into environmental pollution mitigation strategies and the development of efficient bioremediation techniques.

A027

Hydrogenotrophic methanogenesis arising in anoxic carbonate-dominated hyperalkaline conditions.

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Abstract

Historic operation of lime kilns has resulted in significant quantities of calcium oxide-bearing waste, which have been disposed to landfill. The percolation of water through these deposits results in a highly alkaline leachate generation (pH > 12). Calcium hydroxide-dominated leachates increase solubility and subsequent precipitation of atmospheric carbon dioxide as carbonates, referred to as tufa. Despite limited availability of gaseous carbon dioxide, there is evidence that microorganisms within these soils include hydrogenotrophic, methanogenic Archaea. This raises the question as to the mechanisms by which these organisms obtain carbon as an electron acceptor for methanogenesis, given carbon dioxide is largely precipitated.

Within the present study we have generated hydrogen supplemented enrichments at pH 11.0 in which tufa ($CaCO_3$) is the sole carbon source, and whilst rates were slower (0.08 moles.day^{-1} CH_4) compared with carbon dioxide supplemented positive controls (1.82 moles.day^{-1} CH_4) there was still evidence of methanogenesis. Shotgun metagenomic sequencing illustrated the presence of hydrogenotrophic methanogenic archaea within these enrichment through construction of both metagenome-assembled genomes (MAGs), including *Methanocalculus chunghsingensis sp.* and metatranscriptomic analysis. Annotation of these MAGs will be coupled to stable isotope probing to try gain a better understanding of how carbonates are able to support hydrogenotrophic methanogenesis in extreme environments.

A028

Genomic characterisation of *Pseudomonas* on food: implications for spoilage, antimicrobial resistance and human infection

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Abstract

Pseudomonas species are common on food, but their contribution to the antimicrobial resistance gene (ARG) burden within food or as a source of clinical infection is unknown. *Pseudomonas aeruginosa* is an opportunistic pathogen responsible for a wide range of infections and is often hard to treat due to intrinsic and acquired ARGs. This study aimed to understand the potential role of *Pseudomonas* on food as a reservoir of ARGs and to assess the presence of potentially clinically significant *P. aeruginosa* strains on food using whole genome sequencing. Non-specific *Pseudomonas* culturing recovered this bacterial genus from 28/32 retail food samples, although no *P. aeruginosa* was identified. The *Pseudomonas* species recovered were not clinically relevant, contained no ARGs and are likely associated with food spoilage. *P. aeruginosa*-specific culturing recovered this species from 14/128 retail food samples; isolates contained 4-7 ARGs and belonged to 16 sequence types (STs), four of which have been isolated from human infections. Food *P. aeruginosa* isolates from these STs demonstrated high similarity to human-derived isolates, differing by 41-312 single nucleotide polymorphisms. The most frequent *Pseudomonas* recovered from food in this study carried no ARGs and are more likely to play a role in food spoilage rather than infection. *P. aeruginosa* isolates likely to be able to cause human infections and with multidrug resistant genotypes are present on a relatively small but still substantial proportions of retail foods. Given the frequency of exposure, the potential contribution of food to the burden of *P. aeruginosa* infections in humans should be evaluated more closely.

A029

Bacterial detectives: investigating the fabric presence and type on grave soil necrobiome dynamics

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Abstract

The unique and ubiquitous nature of the microbiome has garnered interest for forensic applications such as: geolocation, bodily fluid and personal item identification. Upon death, the successional necrobiome can provide considerable insights. Measurement of subsurface grave soil has proven a successful means of discriminating burial locale and post-mortem (PMI) / post-burial (PBI) interval. Further knowledge development is pertinent to address current gaps prior to implementation to formulate forensic models. One key variable is the presence and type of carrion-associated fabric, with the understanding that clothing influences decomposition atrophy, entomology and movement of cadaveric fluids. We collected grave soil from 15 *ex-situ* laboratory-based murine decomposition microcosms associated with three different fabric types over 170 days. Restriction fragment length polymorphism (RFLP) quantified with Hill ecological indices (^{0}D=species richness, ^{1}D, ^{2}D=species diversity) highlighted temporal clustering of soils, irrespective of fabric type when principal component analysis (PCA) was applied. However, control soils could not be consistently discriminated. High-resolution metabarcoding (16S rRNA) recorded temporal class – and order-level bacterial markers at class such as *Spirochaetia* (10.2%), *Sphingobacteria* (5.2%), *Legionellales* (1.5%), and *Saprospirales* (1.8%) at PBI=32 of advanced decay. Family- and genus-level taxonomic resolution highlighted measurable increases in *Pseudomonas* PBI=8 in cotton- (8.7%) and polyester- (18.3%) wrapped carrion microcosms. Thus, fabric presence and type were reflected in the simulation grave soil necrobiome. However, this study questions the efficacy of *Mus musculus* or mammalian proxies and simulation decomposition microcosms due to ethical constraints, in conducting applicable forensic research in lieu of human taphonomy facilities.

A033

AgMicrobiomeBase.org: A Data Portal for Crop Microbiomes

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Abstract

The UK Crop Microbiome Cryobank aims to provide a comprehensive biological and bioinformatics resource, integrating genomic data with a cryro-preserved soil rhizosphere of major UK crops, barley, oats, oilseed rape, sugar beet, fava beans, and wheat. The crops were grown in agricultural soils from nine different UK sites. The data portal, AgMicrobiomeBase.org, serves as an access point for the sequencing data derived from the microbiomes of crop rhizospheres, along with comprehensive metadata for plant and soil types. The project is in development, with some data already accessible. In addition, approximately 36,000 bacterial isolates have been collected from this project. These isolates are in the process of being sequenced. Users can access the sequence files from the database, which links to the European Nucleotide Archive (ENA) and the MGnify microbiome sequence browser. This dataset serves as a baseline, enabling users to delve into the microbiomes of specific crop-soil combinations and conduct their analyses. A goal is for users to incorporate their own or other publicly available sequencing data for further comprehensive analysis. The project is designed to meet the multiple end-users, including academia, policymakers, and industry professionals, who have a vested interest in sustainable development and food security. The primary aim of the project is to establish a legacy by promoting further research and discovery in the field of microbial ecology. This is achieved through the provision of open access to datasets and resources, highlighting the significance of such access in fostering scientific discovery.

A034

IMADGENN: Using Surrogate Organisms to Assess the Dispersal of Respiratory Droplets and Aerosols Across Individuals, Activities, and Mitigation Strategies

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Abstract

Simulation studies investigating the transmission of respiratory pathogens are challenging to perform and often offer limited insights into the potential infectivity of detected particles. To address this, we assessed the use of normal upper respiratory tract bacteria as indicators for respiratory pathogens. Using a purpose-built Isolator to Measure Aerosol and Droplet GENeratioN (IMADGENN), we examined how respiratory dispersal differs with individual, activity and mitigation strategy.

Healthy volunteers participated in respiratory activities with or without face coverings. We assessed respiratory droplet and aerosol dispersal through passive and active air sampling and identified presumptive respiratory isolates using MALDI-TOF.

Large inter-individual variability was observed. In cohort 1 (n=15), 13% of participants generated 60% of the respiratory bacteria recovered from droplets. Similarly, 20% of participants generated 79% of the bacteria recovered from the air. Wearing a face covering during respiratory activities significantly reduced droplet and aerosol generation. During speech, and regardless of individual (n=16), heightened vocal effort significantly increased the deposition of respiratory droplets and each decibel increase correlated with a 34% higher likelihood of retrieving more airborne bacteria (95% CI: 1.06, 1.70).

This study highlights how individuals differ in terms of their dispersal of respiratory droplets and aerosols, emphasizing the concept of super-spreaders. The results suggest that the impact of performing an activity that increases droplet and/or aerosol generation is more pronounced when conducted by high dispersers. Similarly, when wearing face coverings, the extent of droplet and aerosol reduction depends on the wearer, with the most significant impact observed when worn by ‘super-spreaders’.

A035

Screening of filamentous fungi and their potential for sustainable poly (ε-caprolactone) biodegradation

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Abstract

Plastic is a widely used material in daily life, resulting in increased plastic waste. The use of bioplastics has received attention, which can reduce the environmental pollution caused by non-degradable bioplastics. One of the most popular bioplastics is poly (ε-caprolactone) (PCL), which is a biodegradable polyester and can be readily degraded by microbial lipase and esterase. In this study, we isolated and screened the most efficient fungal species from the air, soil, and pigeon-dropping samples for PCL degradation using the agar diffusion method. The bioplastic films were then tested for degradation in a liquid medium, and the PCL-degrading enzyme was measured using the turbidity method. Lipase activity was measured using p-nitrophenyl substrates. In addition, selected fungal isolates were identified based on morphological characterization and ITS, 18S rRNA and 28S rRNA gene sequencing analysis. The result showed that only 22 isolates from 46 fungal isolates can degrade PCL. Fungal isolate PIN10 had the highest PCL biodegradability, with 84% PCL film weight loss and PCL-degrading activity as 0.036±0.0079 U/mL, while PKF65 had the highest lipase activity with 80.56±3.2937 U/mL. The PCL film surface was uneven and porous after cultivating with isolate PIN10 due to the fungal can produce PCL-degrading enzyme to break down plastic. The strain was identified and most closely related to *Alfaria dandenongensis* CBS 143399^{T}. This research highlights the fungal screening for the biodegradation of PCL and our results are useful for measuring and predicting the degradation rates of PCL films by microorganisms in natural environments.

A036

Investigating the chicken gut microbiome to understand *Campylobacter* prevalence

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Abstract

Abstract

Chickens are a key protein source for humans, yet their microbiome contains bacteria that can be pathogenic to humans. *Campylobacter* is present within the chicken gut, is one of four key global causes of diarrhoeal diseases, and is the leading cause of bacterial foodborne gastroenteritis within humans worldwide.

As poultry demand continues to rise, it is important to provide healthy chickens with as low *Campylobacter* prevalence as possible to reduce the risk of transmission to human. Characterising the changes within the chicken gut microbiome is essential to understand microbial population dynamics and the interlink with *Campylobacter*. To investigate the driving forces for microbial change within the chicken gut microbiome over time, and how this relates to *Campylobacter* prevalence within a natural habitat setting, shotgun metagenomics was performed on selected days and invested using bioinformatics and statistical analysis methods.

We performed a comparative longitudinal day-to-day study between the chicken caeca, the duodenum, small and large intestines. Our statistical analyses initially investigate microbial ecology metrics such as alpha diversity and beta metrics to analyse microbial diversity over time and between organs. We also applied CODA lasso for compositional comparison between performance parameters; mean body weight (BW_mean), body weight gain (Gain), feed intake (FI), feed conversion ratio (FCR) and days. Further investigation is yet to be conducted to better understand these differences.

A037

The impact of Eurasian beaver dams on temporal and spatial variation in concentrations of waterborne microbial pollution

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Abstract

Globally, freshwater environments are under threat from point source and diffuse pollution, habitat loss, and climate change. Enhancing water quality and reducing microbial pollution in freshwater habitats are priorities to reinstate multiple ecosystem services. Over the last decade, beavers have received increasing attention for their role as ecosystem engineers, with proven benefits to aquatic ecosystems, including improved water quality and flow regulation. Here, we quantified the impact of damming by Eurasian beaver (*Castor fiber*) on the concentration of *Escherichia coli* in a stream network. Water samples were collected on 10 dates over two years for a sequence of 14 beaver dams and their pond impoundments to determine the flux of natural *E. coli* populations. Ponds acted as either a source or a sink for *E. coli* depending on the season; however, on average, dam structures were a weak source while ponds were a moderate sink, indicating that sequential dams will act as a net sink of *E. coli*. To simulate an *in-situ* pollution event, a slurry of livestock manure (25 L) was added to two adjacent streams, one beaver-engineered, the other not (control), and waterborne *E. coli* quantified by culture-dependent methods. *E. coli* was strongly attenuated in beaver pools, which reduced peak concentrations but slowed the flushing of *E. coli* through the network compared to the control site. Our findings indicate that beavers can decrease microbial pollution reaching downstream receptors, thus the use of beaver dams or their analogues could support environmental management strategies as part of a suite of nature-based approaches.

A038

Exploring Deep Ocean Microbial Chemical Space atAbyssal Depths: Co-culturing Rare Actinomycetes fromthe Clarion-Clipperton Zone (CCZ) and the PorcupineAbyssal Plain (PAP) for Novel Natural ProductsDiscovery

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Abstract

Antimicrobial Resistance (AMR) remains a global concern, which has led to the exploration of unconventional environments for innovative solutions. The deep ocean is an uncharted frontier for biodiscovery which holds immense potential. As such, studying even well-investigated taxa, such as the bacterial phyla Actinomycetota from these environments has yielded novel biodiversity. This study focused on exploring deep ocean sediments to uncover rare Actinomycetota yielding novel biosynthetic genes and metabolites. Sediment cores from the CCZ and PAP were collected for research purposes, and Actinomycetota were selectively isolated from these samples. The strains were genome sequenced and subjected to metabolomic characterization to identify those with distinct genetic and metabolomic profiles. The isolated strains, possessing between 15 and 38 Biosynthetic Gene Clusters (BGCs), were prioritized for co-culture. One strain's extract molecular network revealed 45 nodes exclusive to its profile, while another exhibited 30 BGCs with low similarity to known clusters, emphasizing its potential for biosynthetic novelty. In this work we hypothesis that co-culturing strains from underexplored geographical locations will enable the discovery of novel natural products. Future work involves selecting CCZ-strains with silent BGCs together with ones presenting a unique metabolome and co-culturing them on diverse culture media to unlock the potential of silent BGCs, exploiting the microbial natural products chemical space and contribute to advancing microbial chemistry in the fight against AMR.

A039

Surveillance and environmental drivers of *Mycobacterium* species

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Abstract

The 190 described species of *Mycobacterium* include members of the *Mycobacterium tuberculosis* complex that are significant human and animal pathogens, and environmental non-tuberculosis mycobacteria (NTM), an increasingly important cause of opportunist infections. Exposure to NTM can also affect BCG vaccination efficacy and the sensitivity and specificity of TB diagnostic tests. This PhD focuses on surveillance of environmental *Mycobacterium* species on farms with persistent problems with bovine TB to understand the role that these species may play in subverting existing TB control measures. At a national level, metagenomic data from wastewater samples collected at multiple sites across Wales will be analysed to assess *Mycobacterium* species and abundance in relation to environmental factors. At a local level, samples will be collected from different sites on 15 Welsh cattle farms, such as water troughs, feed troughs and slurry, that might pose a high risk for cattle of exposure to environmental mycobacterial species, and we will screen these for *Mycobacterium* species using a metagenomic approach. Environmental samples will also be collected before and after biosecurity measures are implemented to assess their impact, and in different seasons to understand climatic effects. Findings will be integrated with farm management data and local wildlife ecology to understand environmental drivers of TB infections. Subsequently, the effect of environmental *Mycobacterium* species on key bovine tuberculosis diagnostic tests, such as the tuberculin skin test, will be assessed using existing bTB skin test results and abattoir surveillance data.

A040

Stress responses elicited by low intensity proton beam irradiation in Aspergillus nidulans model organism

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Abstract

Most of the galactic cosmic radiation consists of high-energy protons, presenting formidable shielding challenges. Short-duration Solar Particle Events (SPE) are relatively easier to shield against, yet they still entail risks for radiation sickness, cancer, and degenerative diseases in biological systems. To better understand the consequences of space-radiation, *Aspergillus nidulans* cultures underwent proton irradiation with 13 MeV H^+ ions (PI) simulating SPE and genome-wide transcriptional changes were recorded. The upregulated gene set was enriched in genes for "DNA-repair", "Telomere maintenance" and "Mitotic DNA integrity checkpoint signaling", suggesting PI caused severe problems in maintaining DNA integrity as expected. The downregulated gene set was enriched in genes related to vegetative growth, which was accompanied by upregulation of the "Negative regulation of the mitotic cell cycle" genes and impaired growth. Antioxidant enzyme genes were also enriched in the downregulated gene set, suggesting that PI induced reductive rather than oxidative changes. Consistent with this observation, genes involved in glutathione synthesis and the oxidative pentose-phosphate shunt were downregulated in PI cultures. Not surprisingly, PI increased the susceptibility to menadione sodium bisulfite (MSB) as an oxidative stress agent. Pretreatment with MSB increased, whereas deletion of the oxidative stress response transcription factor gene *atfA* decreased the fungal tolerance to PI. Therefore, it appears that down-regulation of antioxidant enzymes is not an adaptive response to PI and may represent an Achilles' heel for the fungus coping with radiation. Our data may help to develop better strategies to prevent the rapid spread of fungi in space vehicles.

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A042

Reusing root channels of cover crop monocultures and mixtures enhance the rhizosphere bacterial abundance and functionality

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Abstract

Cover cropping is a successful strategy for safeguarding agricultural soils against erosion, allowing more nutrient and moisture retention, stimulating beneficial microbial activities, and preserving soil structure. As the root channels of winter cover crops are reused in conjunction with maize (*Zea mays* L.) roots during the summer, this allows the cash crop to access resources from deeper layers of the soil horizon. Our work aims at examining the impact of reusing winter cover crop root channels for maize cultivation on the bacterial composition and functionality in the rhizosphere. 16S rRNA gene amplicon sequencing and metaproteomics techniques were used for the study.

Significant differences were observed in the bacterial community composition considering the different cover crop variations, soil profile depths, and maize growth phases. Root channel re-usage has resulted in increasing community abundances, which got elevated after increasing the number of cover crops from monocultures to mixtures. The combination of legumes with both brassicas and grasses displayed improvements for various stages of the carbon cycle (C) and the nitrogen cycle (N). The legumes and brassicas have deeper root channels compared to grasses, which is also a factor for higher bacterial 16S rRNA gene copy numbers and community functions in the different subsurface regimes due to more exudates being secreted by maize roots. Overall, reusing root channels, whether they are mixtures or monocultures, improved metabolic activities in the important carbon and nitrogen cycles and enlarged bacterial communities. This benefits both the soil rhizosphere and the growing crops.

A044

Multiple bacteriocin producer *Lactobacillus gasseri* LM19 as a modulator of the human gut microbiome

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Abstract

The human gut microbiome plays an important role in health and disease, with dysbiosis linked to illnesses such as inflammatory bowel syndrome, inflammatory bowel diseases such as ulcerative colitis and Crohn's disease as well as obesity and diabetes. Bacteriocins are bacterially produced, ribosomally synthesized antimicrobial peptides. They have been proposed for use as novel antimicrobials to aid against antimicrobial resistance. We are exploring their potential as modulators of the human gut microbiome, allowing us to shift microbial populations towards healthier outcomes. Lactic acid bacterium *Lactobacillus gasseri* LM19 produces multiple bacteriocins. In co-culture experiments of *L. gasseri* LM19 with synthetic and faecal gut microbiome communities we utilized qPCR and metabolomic and metagenomic analysis to study the effects of *L. gasseri* LM19 on population composition as well as the effect of the microbiome itself on bacteriocin gene expression. Gene expression analysis demonstrated the up-regulation of all six bacteriocin-encoding genes during the late log phase of bacterial growth. This correlated with an increase in antimicrobial activity in the culture supernatant. No antimicrobial activity was observed against members of the extended Simplified Human Intestinal Microbiota (SIHUMIx) consortium whilst activity was seen against *Lactobacillus delbrueckii subsp. bulgaricus*. The SIHUMIx consortium is a representation of a 'healthy' human gut microbiome that is useful for studying bacteriocin production. Our next step is to demonstrate bacteriocin production in a complex colon model that closely mimics the ecology of the human gut microbiota.

A046

Evolutionary dynamics of wild bacterial communities

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Abstract

Bacteria perform a multitude of biological functions which drive key ecosystem processes. Therefore, understanding how bacteria respond and adapt to environmental change is key to our understanding of ecosystem functioning and resilience. Many studies have shown that single strains of bacteria can rapidly adapt to new conditions in the lab, but it is unclear whether this adaptive capacity is an important mechanism in the response of natural microbial communities to change. Indeed, growing evidence suggests that evolution depends as much on the community of interacting species as the properties of the species in question. Furthermore, communities can respond to change in alternative ways to genetic adaptation, such as sorting of extant diversity or immigration of new species. Here using a combination of field and lab experiments, employing functional measures and metagenomics approaches, we investigate the importance of genetic adaptation in the wild. We ask what proportion of the response to environmental change is due to local evolution and local ecological sorting of species versus the influx of new genotypes and new species.

A047

Characterization of Penicillium roqueforti Isolates from Turkish Traditional Blue Cheeses: Technological Properties and Volatile Compounds

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Abstract

Penicillium roqueforti is the principal filamentous fungus responsible for the blue-green color in Turkish blue cheeses like Konya Kuflu Tulum and Erzurum Kuflu Civil during the ripening process. This study aims to determine the technological properties and volatile compounds of *P. roqueforti* isolates obtained from Turkish blue cheeses. In previous studies, 120 *P. roqueforti* isolates were acquired from traditional Turkish blue cheeses (n=61). From these, 20 isolates representing the population based on sequence types determined by microsatellite analysis were selected for this study. The mycelium growth, salt resistance (at 1%, 3%, 6% NaCl), proteolytic, and lipolytic activities of the isolates at 12°C and 25°C were assessed. Colony diameters ranged between 29-77.2 mm on MEA without NaCl, while at 1% NaCl, they ranged from 36-74.7 mm. With increased NaCl concentration (3% and 6%), colony diameters reduced to 37.5-68.5 mm and 19.5-49.7 mm, respectively. After seven days of incubation at 25°C on mycological agar containing 10% skimmed milk, 10 isolates showed a clear zone, indicating proteolytic activity. Lipolytic activity was observed in 16 isolates, presenting an opaque zone around the colonies after incubation on Tween 80 agar at 25°C. The data were analyzed using analysis of variance (ANOVA) and principal component analysis (PCA) through Minitab 18 (version 18.1) software. After the statistical analysis, the volatile compounds of the selected isolates were identified using GC-MS. Detecting *P. roqueforti* strains with distinct characteristics in Turkish blue cheeses suggests the potential use of these strains as secondary starters in cheese production.

A048

Effect of membrane fouling on the removal of antibiotic resistant bacteria and genes from wastewater treatment effluents with ultrafiltration

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Abstract

Wastewater treatment plants (WWTP) are known hotspots for the development of antibiotic resistance. Antibiotic resistance genes (ARGs), genetic elements conferring resistance to antibiotics, are normally found inside bacterial cells. However, during biological treatment, these elements (e.g. plasmids) can be released into the surrounding environment, where they can be acquired by other bacteria via horizontal gene transfer. WWTP effluents become vehicles for the dissemination of not only antibiotic resistant bacteria (ARB), containing intracellular ARGs, but also extracellular ARGs (eARGs) and, therefore, both should be considered when designing preventive strategies.

In this study, post-treatment of secondary effluents with ultrafiltration membranes was investigated to verify the membrane efficiency for removing eARGs and ARB under different degrees of fouling. The feedwater used was a synthetic effluent containing either target eARGs (cell-free extracts of IncW plasmid R388, conferring resistance to sulfonamides) or ARB *(P. putida KT2440* harboring the same plasmid). The lab-scale set-up consisted of cross-flow membrane cassettes (100KDa, polyethersulfone), each operated in batches over 4 weeks, approximately 20 hours of operation divided into 12 consecutive experiments.

Quantification of eARGs and ARB was performed by quantitative polymerase chain reaction (qPCR) and flow cytometry, respectively. Membrane fouling was recorded during operation as permeate flow rate decrease whereas the membrane surface was inspected at the end of the experiments with scanning electron microscopy (SEM). The results showed high removal efficiencies for ARB (99.97% average) and eARGs (99.4% average) regardless of the degree of membrane fouling, proving that membrane technology is a promising alternative to minimize environmental spread of antibiotic resistance.

A049

BIOCONTROL POTENTIAL OF *Bacillus thuringiensis* ISOLATED FROM SOIL SAMPLES AGAINST LARVA OF MOSQUITO

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Abstract

This study aimed at assessing the biocontrol potential of *Bacillus thuringiensis* isolated from soil samples against larvae of mosquito. Soil samples were collected from Imo State University farm and mosquito larvae was collected from stagnant water. Soil samples were analyzed using standard microbiological procedures. Result of the study showed that the suspected *Bacillus thuringiensis* isolates were cream in colour, tend to have large frosted glass appearance, initially, but become opaque and spread over the plates. Some colonies were mucoid in nature, others brittle. The isolates were Gram- positive and spore formers. Results of the biocontrol activity of *Bacillus thuringiensis* against mosquito larvae was more effective at 24hours of incubation at the various dilution factors as it left all five larvae dead. 10^0 dilution factors were the most effective. It left all larvae dead even at 4 hours of incubation. However, 10^{-4} and 10^{-5} were the least effective dilution factors leaving 4 and 5 larvae alive respectively. Conclusively, the results obtained in this study clearly demonstrated the efficiency of the *Bacillus thuringiensis* in controlling mosquito larvae. The use of *Bacillus thuringiensis* as a biocontrol agent against mosquito larva is preferred as it is environmentally friendly and does not deplete the ozone layer unlike the regular pesticides used in killing mosquitoes in most communities.

A051

Evaluating the efficacy of antimicrobial materials under varying environmental conditions

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Abstract

The use of antimicrobial materials (AMMs) in the built environment is increasingly used to control microbial survival and growth. Standardised test methods offer a reproducible approach to efficacy assessment of AMMs. However, many standardised methods (e.g. ISO 22196) do not assess efficacy under environmental conditions that could be considered realistic. Instead, conditions such as relative humidity above 90% are used, which keeps materials wet for longer than they would be in-use. Copper surfaces were assessed for antibacterial, antifungal, and antiviral efficacy using a simulated splash method under environmental conditions relevant to the built environment. Copper coupons were inoculated with 10 1 mL droplets of either *E. coli*, *S. aureus*, *C. albicans* or Phi6 phage in 0.15 % bovine serum albumin and incubated at 20±2 °C at varying relative humidity values (Low – 10-20 %, Medium – 45-55 %, high – 80-90 %). Inoculum was recovered in to SCDLP neutraliser immediately (i) after incubation, (ii) at the point of inoculum evaporation, and (iii) two hours post-evaporation. The recovered inoculum was then quantified to count viable numbers. A faster evaporation time for the inoculum was observed at lower relative humidity as well as reduced antimicrobial activity of copper against all microorganisms. Further analysis of the antimicrobial efficacy of copper surfaces is required to determine whether its use on a wide scale can be justified. Alternately, targeted placement of copper surfaces where favourable conditions are expected could aid in maximising effectiveness.

A052

Investigating the Relationship Between Decay Symptoms in *Araucaria araucana* Chilean National Tree and Soil Microbial Communities

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Abstract

Abiotic stress can weaken plants by disrupting their physiological capacity and acting as a predisposing factor. This disruption not only affects the plant's physiology but also impacts the balance of its microbiome, which plays a vital role in enhancing the plant's resistance to stress factors. The diversity and balance of microorganisms in the microbiome are crucial for the plant's health, and any alterations to it can result in symptoms of decline. The stability of the microbiome's functions is closely linked to greater microbial diversity, which improves the plant's ability to respond to stress factors. In 2016, *Araucaria araucana*, Chile´s national tree, exhibited crown decay and death, which were attributed to the bioclimatic stress experienced by the ecosystem. This stress weakened the trees' ability to respond effectively. The objective of this study was to examine the taxonomic and functional diversity of the soil microbiome in asymptomatic *A. araucana* trees compared to those showing severe crown death symptoms. Soil samples from decaying *A. araucana* trees were analyzed to evaluate the composition and diversity of fungal and oomycete communities and their relationship with the disease symptoms. The study found that the taxonomic and functional diversity of the soil in decaying *A. araucana* trees were correlated with the decay symptoms.

A053

A plant-based, eco-friendly, cost-effective stain for live cell imaging

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Abstract

We aim to develop an eco-friendly, economical, plant-based dye to stain live cells. This dye could also have other applications in textile, paper, and other industries. Dyes provide contrast for the visualization of cells under the microscope. Commonly used stains are positively charged basic dyes that bind to the negatively charged cell surfaces and impart a color to the cell. However, a majority of currently used dyes in India and elsewhere are recalcitrant and are not biodegradable. Many dyes are known to cause toxicity to plants and animals and even could be passed on to the food chain affecting public health via the release of dye-loaded wastewater. Hence there is a need to develop biodegradable and cost-efficient that could be used for staining and other industrial uses in alignment with sustainable development goals. India has a rich diversity of dye-yielding plant species. For the proof of concept, *Bauhinia purpurae,* a species of flowering plant in the family Fabaceae, native to the Indian subcontinent is chosen. The natural violet dye of the flowers from this plant was extracted in appropriate solvents and tested for the ability of the dye to stain live cells. Our preliminary data shows that this natural dye could stain a few microbes including *Escherichia coli*. Our goal is to develop this and other dyes into a sustainable, eco-friendly, economical dye that will have widespread applications to stain not only live cells but also many applications in the textile and paper industry.

A056

Multidrug resistant Escherichia coli and Salmonella spp. isolated from a shared biodynamic pig farm

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Abstract

The UK Antimicrobial Resistance National Action Plan takes a One-Health approach to surveillance, encompassing humans, animals, agriculture, and the environment. To complement genomics studies of bacterial flora from different compartments on a single mixed arable and pig farm, phenotypic resistance of *Escherichia coli* and *Salmonella* spp. was determined from 94 samples [water (*n*=24), manure pile (*n*=14), feed (*n*=6), soil (*n*=16), swine faeces (*n*=14), rat faeces (*n*=11) and pork sausage (*n*=9)]. *Escherichia coli* and *Salmonella* spp. were isolated, identified using MALDI-TOF mass spectrometry and tested for antibiotic susceptibility through the Kirby–Bauer disc diffusion method. A total of 52 confirmed *E. coli* (from all sample types) and 2 *Salmonella* isolates (from rat faeces) were obtained over three trips. All *E. coli* isolates from animal feed were multidrug resistant (MDR), with two (40%) isolates resistant to all 10 antibiotic classes tested. MDR was detected in *E. coli* isolates from manure piles (*n*=6), rat faeces (*n*=5), soil (*n*=4), pork sausages (*n*=5), swine faeces (*n*=2), and water (*n*=2). Half of the *E. coli* isolates (*n*=4) from pork sausages were resistant to all antibiotics tested, including critically important antimicrobials (3rd- and 4th-generation cephalosporins) and highly important (aminoglycosides and penicillins). Both *Salmonella* spp. isolates were MDR, with both resistant to gentamicin and tetracycline. Ongoing work, including whole genome sequencing and metagenomic profiling of samples, will help uncover underlying resistance mechanisms and sharing between different compartments. However, the high levels of contamination of both human and animal foodstuffs suggest a need to better understand how AMR circulates within mixed farming systems.

A057

Evaluating the presence of extended-spectrum-β-lactamase-producing and/or AmpC-β-lactamase-producing Enterobacterales within a communal biodynamic farm environment

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Abstract

The emergence of extended-spectrum-b-lactamase-producing (ESBL) and AmpC-b-lactamase-producing (AmpC) Enterobacterales is a challenge to human and animal health. Livestock and environmental sources can serve as conduits for multidrug-resistant (MDR) bacteria. The purpose of this study was to investigate the presence of ESBL- and/or AmpC-Enterobacterales in a mixed arable and pig farm. A total of 94 samples were collected from water (*n*=24), soil (*n*=16), animal feed (*n*=6), swine faeces (*n*=14), rat faeces (*n*=11), manure pile (*n*=14) and pork sausages (*n*=9). Selective enrichment, plating onto chromogenic media, MALDI-TOF mass spectrometry, antibiotic susceptibility (disc diffusion, double-disc synergistic test and AmpC-detection kit) were used to identify ESBL/AmpC-Enterobacterales. Thirty-two ESBL-Enterobacterales were isolated from various samples, with a notable proportion from water (43.8%, *n*=14) and pork sausages (21.9%, *n*=7). Moreover, 29/32 of isolates were ESBL-Enterobacterales and 3/32 were ESBL- and AmpC-Enterobacterales. Multidrug resistance was observed in 78.1% of isolates, with high rates of ampicillin (93.8%), tetracycline (56.3%) and imipenem (43.8%) resistance. Dominant ESBL- and/or AmpC-Enterobacterales identified included *Serratia fonticola* (*n*=11; 6 from pork sausages, 3 from water and 2 from soil) and *Proteus vulgaris* (*n*=11; 4 from manure pile, 3 from swine faeces, 2 from water, 1 from rat faeces and 1 from pork sausages. The three ESBL-*Escherichia coli*, one ESBL-*Klebsiella pneumoniae* and one ESBL-*Citrobacter freundii* were isolated from water. These findings highlight the importance of investigation of MDR within the total agricultural environment and improved comprehension of the factors that promote selection for resistance across all farm compartments.

A058

Development of a method for isolating non-tuberculous mycobacteria from potable water

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Abstract

Non-tuberculous mycobacteria (NTM) are natural inhabitants of water that can cause serious opportunistic infections in immunocompromised individuals. Environmental sampling identifies potential sources of infection, however there is currently no standard method for isolating NTM from potable water.

Different sample processing methods were compared. Variables included sample bottle (+/- sodium thiosulphate); storage temperature (ambient vs 4°C); storage duration (24-72 hours); decontamination treatment (+/- cetylpyridinium chloride (CPC), N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH)); culture media (Middlebrook 7H11 vs NTM Elite agar) and incubation temperature (30°C vs 37°C). Impact on NTM recovery was assessed using artificially spiked water (*Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium chimaera*, *Mycobacterium abscessus* and *Mycobacterium chelonae*) and naturally colonised water samples.

Storage time broadly reduced recovery of NTM, whilst effects of bottle type and temperature varied depending on species. Storage had least impact when dosed bottles were refrigerated for ≤24 hours. Decontamination using CPC or NALC-NaOH reduced NTM concentrations in spiked samples variably depending on species (0-99.7% and 26-100% reduction respectively compared to no decontamination). Neither treatment entirely prevented culture of non-NTM species. Culturing directly onto NTM Elite agar effectively reduced non-NTM contamination and impacted NTM recovery from spiked samples less (0-45% reduction). 30°C incubation allowed growth of NTM species not cultivated at 37°C. At 37°C, overgrowth of rapid-growing NTM was reduced, improving detection of slow-growing NTM.

We recommend isolating NTM from potable water by collecting samples in sodium thiosulphate dosed bottles; if immediate processing is not possible, keep refrigerated for ≤24 hours before culturing on NTM Elite agar at 30°C and 37°C.

A061

Identification of Irish Cattle as potential reservoirs of fluoroquinolone resistance, extended spectrum β-lactamase producing Enterobacteriaceae (ESBL), and carbapenemase producing Enterobacteriaceae (CPE)

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Abstract

AMR is very prevalent within the agriculture sector especially within food producing animals which are involved in intensive production. The careless use of antimicrobials has contributed to the extensive dissemination of AMR bacteria within animal adjacent environments. This practice has led to an unprecedented rise in AMR and multi-drug resistant (MDR) bacteria. As the public health threat of AMR reaches crisis point, the future of routine antibiotic use becomes untenable. The study aims to assess-

1. The prevalence of the CPE, ESBL Fluoroquinolone within Irish cattle
2. The ability of the AMR Enterobacteriaceae to persist in slurry samples exposed to winter and summer conditions

The detection of AMR and MDR was split phenotypic and genotypic methods. Both methods of analysis were employed within the study including, selective agar, antimicrobial susceptibility testing (Kirby-Bauer), MALDI-TOF MS, and PCR. The samples analysed were fresh faecal and slurry sourced from Irish cattle herds, both dairy and suckler.

Initial phenotypic findings have detected CPEs (22.3%), ESBL (21.8%) and fluoroquinolone (5.8%) resistance within slurry and fresh bovine faecal samples (N=175). Many of these samples displayed MDR (6.7%). The summer slurry samples demonstrated AMR for 3 months and winter samples continue to display AMR.

Enterobacteria have been classified by the WHO as severely drug resistant bacteria due to extensive production and dissemination of CPEs and ESBLs which supports these initial results. As this is the first study of its kind within Ireland, these are worrying results as MDR may be more prevalent than first anticipated.

A062

CHARACTERISATION OF THE FUNCTIONAL CHANGES IN THE CORALLIUM RUBRUM MICROBIOME DURING THERMAL STRESS

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Abstract

Corals serve as a model for investigating changes in the host-associated microbiome in response to stress. As holobionts, corals harbor diverse microbial communities that are thought to be critical to their health and adaptation to environmental change. However, we lack information on their actual biological function, as most studies focus only on changes in microbial identity and abundance.

While the microbiome of zooxanthellate corals has been well studied, it remains largely unexplored in azooxanthellate corals, especially octocorals such as *Corallium rubrum*. *C. rubrum*, together with other gorgonian octocorals, is a foundational species of coralligenous reefs. They are increasingly threatened by human activities and global warming, which emphasizes the urgency of understanding how they respond to environmental stress. One of the predominant bacterial species associated with Mediterranean gorgonians belongs to the genus *Endozoicomonas*, whose functions are still being unraveled. However, *C. rubrum* is an exception, as it is dominated by bacteria of the Spirochaetaceae family, whose functions in *C. rubrum* are still unknown.

In this context, our study focuses on the metatranscriptome of *C. rubrum* under thermal stress and hypothesizes that high temperatures may alter the functionality of bacterial symbionts, particularly Spirochaetes, potentially leading to coral death. An experiment was conducted in which the corals were exposed to ambient (15°C) and an elevated temperature (24°C). Functional analysis of the bacteria revealed changes in some metabolic processes, associated with Alphaproteobacteria, Gammaproteobacteria and Spirochaetota. *C. rubrum* metatranscriptome analysis under thermal stress provides insight into the involvement of bacteria in coral health.

A063

Genomic epidemiological surveillance of a cholera outbreak in northern India.

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Abstract

Cholera has existed in India for centuries. The freshwater environment of North India especially in and around the territory of Chandigarh witnesses' seasonal cholera occurrences between July and October, marked by outbreaks and sporadic cases. Annually, 1 to 3 outbreaks are reported in the area, making it a cholera hot spot. This study focuses on the intra-outbreak dynamics and source identification of a severe cholera outbreak that occurred in September 2019 in Shahpur village, situated in the Kalka region, Haryana. The outbreak predominantly affected migrant laborers and their families in unlicensed huts, with an 8% (196 out of 2,602) attack rate and a 1% (2 out of 196) case fatality rate. Tube-well water emerged as the sole water source for routine activities in the affected area. High-throughput genome sequencing of 73 isolates (18 clinical, 55 environmental) revealed the presence of 22 strains belonging to the current pandemic lineage 7PET only in stool and in-house stored water from index cases. In contrast 51 diverse non-7PET strains were found in environmental samples including tube-well water and asymptomatic individuals, indicating a broader reservoir and potential outbreak sources. In summary, our study underscores the crucial impact of water storage and distribution systems on the transmission of cholera in high-risk areas. It shows that integration of epidemiological investigations with genome sequencing yields essential insights, offering guidance for targeted interventions aimed at effectively controlling cholera outbreaks in local regions.

A064

The airborne survival of vancomycin-intermediate-resistant *Staphylococcus aureus*

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Abstract

Since the introduction of vancomycin to treat methicillin-resistant *Staphylococcus aureus*, the emergence of vancomycin-resistant *Staphylococcus aureus* has become an increasing problem in clinical settings, particularly within immunocompromised populations. Recent evidence in vancomycin-intermediate resistance *S. aureus* (VISA) has suggested that mutations in operons associated with cell wall synthesis (*walKR* and *graS*) can increase cell wall thickness – a potential defence against oxidative stress during aerosolization. We aimed to determine the impact of the thicker cell wall of VISA on aerosol survival, using the controlled electrodynamic levitation and extraction of bioaerosol onto a substrate (CELEBS) system. Bacterial viability post-aerosolization in the CELEBS instrument was confirmed for both VISA (strain M1150) and wild-type *S. aureus* (WTSA) in Mueller-Hinton (MH) broth at various relative humidity (RH) levels (30%, 55%, 90%).

M1150 exhibited sustained viability at all tested humidities at 10, 20 and 60mins, with statistically significant greater viability than WTSA particularly at 55% and 90% RH ($p<0.05$). Whilst WTSA survived best at 30% RH, by one hour viability decreased significantly when compared to M1150 ($p=0.018$), which had a 40% greater mean survival. Further investigation into the role of oxidative stress in aerosol phase was conducted by studying catalase negative SA, other respiratory bacteria including *Neisseria* spp, and by using respiratory fluid surrogates.

The aerostability of this VISA strain, particularly at intermediate humidity typical of hospitals, suggests protection from oxidative stress during the aerosol phase and a potential mechanism of transmission. Therefore, thorough containment and investigation of nosocomial VISA outbreaks should include air sampling and appropriate ventilation.

A065

'Comparison of Resistant and Biofilm Forming *Escherichia coli* in Flooded and Non-Flooded Agricultural Soil'

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Abstract

Background: Antimicrobial resistance and infectious diseases associated with climate change require a One Health approach. Ireland will likely experience increased flooding due to heavy rainfall events impacting agricultural land, leading to increased risk of infection, due to *Escherichia coli* and other *Enterobacteriaceae.*

Methods: Agricultural sites (n = 8) were identified and soil samples were taken from flooded and non-flooded areas. Through enrichment, *E. coli* were selected using EMB agar. 16S rRNA PCR was used to determine *E.coli* identity. Disk diffusion and minimum inhibitory concentration methods were used to test antibiotic susceptibility. To test biofilm formation, 96-well plates and crystal violet staining have been utilised.

Results: A greater number of *E. coli* (n = 46) were isolated from flooded soil than non-flooded soil (n = 14) Almost all (n = 13) *E. coli* isolates from non-flooded soil were susceptible to all antimicrobials investigated. 1 isolate was ampicillin resistant. In flooded soils, 11 isolates exhibited resistance to ampicillin, tetracycline, ciprofloxacin, trimethoprim, sulphonamide and trimethoprim/sulfamethoxazole. Resistance to gentamicin, kanamycin and azithromycin were also detected. Preliminary results of biofilm assays show 73.33% of *E.coli* tested (n = 15) to date can form biofilms. Of these, 6 are good biofilm formers while 5 are poor.

Conclusion: A greater number of *E.coli* were isolated from flooded than non-flooded soils, 46 and 14 respectively, suggesting flooded soils are potential reservoirs for *E.coli*. *E.coli* from flooded soils displayed greater antibiotic resistance. Preliminary results show many biofilms forming *E.coli* of mixed capabilities.

A066

Production of butyric acid in an anaerobic route using crude hydrolysate derived from lignocellulosic biomass found in North-West Himalayan region .

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Abstract

This research showcases the cultivation of *Clostridium tyrobutyricum* ATCC 25755 and the batch fermentation process to synthesize butyric acid, harnessing both pentose (C_5) and hexose (C_6) sugars extracted from lignocellulosic biomass. The microorganism exhibited specific growth rates of 0.324 h^{-1}, 0.2 h^{-1}, and 0.268 h^{-1} in the presence of commercial glucose, xylose, and a mix of sugars, at concentrations of 60, 30, and 40 gL^{-1} respectively. The ideal pH for both growth and butyric acid synthesis was identified as 6.0. The peak of butyric acid yield reached 25.09 gL^{-1}, with a productivity rate of 2.10 $gL^{-1}h^{-1}$ under the optimal conditions of a pH of 6.0, an acetate concentration of 10 gL^{-1}, and a yeast extract concentration of 6 gL^{-1}, utilizing lignocellulose-sourced sugars. The kinetic modeling through the Leudeking-Piret equation indicated a pattern of mixed-growth associated product formation, with constants β (0.165 g of butyric acid per g of cells per hour) and α (25.72). A higher butyric acid concentration was achieved using unrefined lignocellulosic hydrolysate, which suggests potential cost savings in downstream separation process for industrial applications. Hence, the study confirms the potential of *C. tyrobutyricum* ATCC 25755 to efficiently produce butyric acid from economically viable and environment friendly lignocellulosic hydrolysates, in addition to conventional glucose and xylose substrates.

A067

Elucidating the mechanism and evolution of the ammonium transporting protein superfamily

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Abstract

Transportation of ammonium across cellular membranes, essential to all kingdoms of life, is facilitated by the Ammonium transporter (Amt), Methylammonium permeases (Mep) and Rhesus (Rh) superfamily of membrane proteins. While being structurally highly conserved, their functions differ: scavenging, concentration sensing, exporting and maintenance of concentration gradients. Fungal Mep proteins trigger pseudohyphal growth which may lead to pathogenesis.

Many cases of horizonal gene transfer fill the family history, though no study focuses on the family evolution as a whole. Doing so may put these cases into context causing a potential rethink. Using the conserved structure of the protein, a tree was made to answer some remaining questions about the origin of these proteins. Did our last common ancestor have one ammonium transporting protein which diverged in specific organisms to fulfil their needs, did it have a number with different functions that were retained or lost in the generations since?

Our lab uses Solid Supported Membrane Electrophysiology to study these proteins. This involves purification, reconstitution into liposomes and subjection to electrophysiological measurements. To bypass low yield and stability of eukaryotic ammonium transporting proteins, we have created vesicles directly from the cells used for overexpression. This will greatly expand the range of proteins available for study.

Using guidance from the evolutionary tree and the vesicles, proteins can be measured from all parts of the family to elucidate whether there is a conserved mechanism that achieves their functions. If successful, it may lead to new therapeutics specifically targeting fungal pathogens through their Mep proteins.

A068

Investigating alternative antimicrobial strategies to treat neonatal S. capitis infections

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Abstract

Staphylococcus capitis (*S. capitis*) is a major pathogen associated with severe neonatal infections. Of particular significance is the NRCS-A clone, which is associated with a multi-drug resistance (MDR) profile. The global prevalence of MDR pathogens continue to rise, highlighting the need for alternative antimicrobial strategies. This study examined the antimicrobial potential of the antibacterial peptides nisin and bovine lactoferrin (bvLf) against *S. capitis* isolates. The activity of wild type nisin (nisin A) and two bioengineered nisin derivatives (PV and K12A-PV) was assessed using deferred antagonism assays and minimum inhibitory concentration (MIC) assays. Data demonstrated that nisin PV had improved activity against the *S. capitis* strains, compared to nisin A.MIC values for nisin PV ranged from 6 to 50 µg/mL compared to values for nisin A which ranged from 12.5 to 50 µg/mL. Combinations of nisin peptides with antibiotics were assessed for synergistic effects using growth curve and time-kill assays. A combination of nisin PV/ampicillin significantly inhibited the growth of *S. capitis*.

Investigating the antimicrobial and antibiofilm properties of bvLf against the *S capitis* isolates demonstrated that at 750 µg/ml, bvLf significantly inhibited ($p< 0.05$) growth of the majority of isolates (90%) and inhibited biofilm formation in all strains. Combining bvLf with = selected antibiotics (penicillin, ampicillin, erythromycin, vancomycin) indicated no synergistic effects for any combination tested.

The findings of this study highlight the potential of nisin peptides and bvLf as alternative antimicrobial strategies for treating infections caused by the neonatal pathogen, *S capitis*.

A069

Potential Human Pathogenic Candida Species on Plastic Pollutants on Public Beaches in Nigeria

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Abstract

Plastic pollution is a growing global concern because of its impact on the environment and human health. Plastic pollutants become rapidly colonised by microbial communities, which can contain viral, bacterial and fungal pathogens that can subsequently become disseminated within the environment. This study evaluated whether species of human pathogenic *Candida* naturally colonise various plastic pollutants at six selected beaches in southwest Nigeria. Plastics were pre-enriched in yeast peptone dextrose broth and species of *Candida* identified on selective chromogenic agar plates, with presumptive human pathogenic *Candida* confirmed by PCR. The susceptibilities of selected isolates to fluconazole were also evaluated. Human pathogenic *Candida* species were isolated from plastic debris collected from all six beaches. This study has demonstrated that human pathogenic *Candida* species can colonise and persist on plastic debris on beaches in tropical climates like Nigeria and could pose a potential human health risk through exposure, particularly if they are expressing anti-fungal drug resistance.

A070

Metagenomic guided bioprospecting of carbon dioxide-driven metabolism, in geothermal springs, with potential for novel bioprocesses

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Abstract

Due to anthropogenic emissions, the atmospheric concentration of CO_2 has risen to more than 50% above preindustrial levels. Many environmental microbiomes have the capability to metabolize CO_2 into biomass and various primary and secondary metabolites. Utilizing consortium-based microbial CO_2 fixation in industrial bio-conversion processes represents an effective strategy for simultaneously reducing anthropogenic emissions and synthesizing value-added bio-products. Environments such as geothermal springs are known to host a diverse range of microbial autotrophs that fix CO_2 as a source of carbon. To investigate the metabolic potential of environmental consortia for CO_2 valorisation, we conducted a global comparative analysis using experimentally acquired and publicly available metagenomic datasets from geothermal springs. A custom workflow for community-level analysis identified key taxa, CO_2 fixation pathways, and genes involved in bioproduct synthesis. Temperature was implicated as driving force for selecting key taxa, carbon fixation pathways, and bioproduct biosynthesis. By employing a combination of gene- and taxonomic-focused approaches, we uncovered global trends in microbial activity in geothermal springs. These trends facilitate the understanding, assembly, and domestication of microbial communities for the conversion of CO_2 into value-added bio-products.

A072

Biofilm community structure dynamics on plastics following transmission through WWTP

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Abstract

Wastewater treatment plants (WWTP) are hubs for collection and dissemination of plastic to and from the environment. WWTP remove up to 99 % of plastic using primary, secondary and tertiary treatment, however small amounts are still released into the environment causing significant ecological and environmental impact. WWTP effluent discharges directly into the sea or into rivers, where waste travels through freshwater and brackish water before entering the marine environment. Once here, dispersal rate and distance increase. The ability of plastic to form unique ecological niches, harbouring wastewater microbes and protecting them from changing abiotic conditions allowing persistence in the marine environment is of current concern.

To investigate plastic community structure dynamics we exposed high density polyethylene, low density polyethylene, polyethylene terephthalate and polypropylene to a series of mesocosms containing wastewater effluent, downstream river water, brackish water and seawater, simulating relevant residence times in each, for a total of 16 weeks. Using 16S rRNA gene sequencing and whole metagenome sequencing we assessed the community changes finding that; (i) wastewater effluent associated microbes are replaced by typical marine taxa within 1 week of seawater exposure, (ii) statistically significant changes to community structure take place within 1 week of seawater exposure and again after 3 weeks and (iii) some taxa show a preference for certain plastic types.

A074

Immobilization of Stenotrophomonas bentonitica BII-R7 within calcium Na-Alginate hydrogels for Se(IV) bioremediation and Se-NPs synthesis within the context of circular economy.

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Abstract

Uncontrolled selenium (Se) release into the environment leads to pollution and increases the threat of Se intoxication in organisms. Bioremediation is a current used technology in removing metalloids like Se. To create cost-effective, efficient, and non-polluting carrier systems, natural hydrogels have been utilized as immobilization matrices because they are biocompatible, abundant, and non-polluting and the fact that immobilized cells are more effective than planktonic cells.

In this work, we immobilized *S. bentonitica* BII-R7 cells, within calcium sodium-alginate hydrogels to develop a novel eco-friendly Se removal method. Alginate and cells solutions were mixed and gelled into $CaCl_2$ resulting bead-shaped bio-hydrogels.

Selenium removal capacity was studied incubating beads in Luria Bertani medium (LB) at 100% (LB100) and 10% (LB10) with the addition of Se(IV). LB100 showed a 70% removal rate while LB10 exhibited only 20%. Compressive rheology studies revealed that beads incubated in LB100 were deteriorated by the end while, beads incubated in LB10 did not. Scanning Elecronic Microscopy (SEM) of the bead surface and inner part showed extensive bacterial growth on the surface and scarce growth inside the matrix and Se-NPs were encountered in both parts. Physicochemical properties of the Se-NPs were analysed observing cross-section cuts on High-Resolution Transmission Electronic Microscopy (HRTEM).

This work highlights the great potential of the developed system for Se(IV) removal. Nonetheless, further work is needed to achieve a balance between bio-hydrogel stability and Se(IV) reduction rates.

Acknowledgments

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A075

Limiting dietary iron at weaning improves gut bacterial populations without affecting weight gain in piglets

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Abstract

Post-weaning diarrhoea (PWD) is the primary welfare and economic concern in pig farming and is minimised by zinc oxide (ZnO) treatment, which is being banned. Piglets receive an iron injection at birth and high dietary iron at weaning to prevent iron-deficiency anaemia. Increases in luminal iron provide a favourable environment for enteric pathogens and can exacerbate PWD. Furthermore, the microbial gut community drives immune development which skews in the presence of pathogens. However, piglets are not anaemic at weaning, so we hypothesised that dietary iron could be reduced around weaning without detrimental consequences. Eighteen 28-day old piglets were allocated into sex-balanced litter-matched treatment groups (n=6): High iron with ZnO, high iron and low iron, without ZnO. Growth and iron status was monitored, faecal samples and intestinal tissues were taken. Microbial populations were assessed by 16S rDNA sequencing and Qiagen CLC genomics was used to perform OTU clustering and diversity analysis. Intestinal sections were analysed using 4-colour quantitative fluorescence immunohistology to quantify immune-associated protein expression. Piglets provided with low dietary iron had increased *Roseburia* (p<0.05) and reduced *Staphylococcus* (p<0.01). Whereas piglets given ZnO had reduced *Campylobacter* (p<0.01), but increased *Bifidobacterium* (p<0.02). Higher dietary iron reduced expression of immune markers in comparison to piglets given ZnO or low oral iron. No significant differences were reported in weight gain or iron status between treatment groups. The reduction in dietary iron benefitted the gut microbial profile without reducing weight gain, iron storage or immunity and therefore provides a novel strategy to reduce PWD.

A076

Insights into the characteristics of a novel strain of *Enterobacter* spp. in detergent degradation in grey water and plant growth promotion in *Solanum melongena*

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Abstract

The study aims to identify a bacterium with detergent degradation capability and potential plant growth-promoting properties to reuse detergent-polluted water for irrigation. The bacterium was isolated from the soil using a basal medium with 0.05% sodium dodecyl sulfate (SDS) as the sole carbon source. The bacterium showed positive results for potassium and phosphate solubilization. The 16S rRNA sequence showed 99.71% similarity with three other *Enterobacter* species by BLASTn and a phylogenetic tree was constructed. The newly identified bacterium was named *Enterobacter* spp. strain MSK86 with the accession number OR398804. The bacterium was also found to be positive for zinc and calcium solubilization, ammonia, indole acetic acid, and siderophore production and showed antagonism to *Aspergillus niger* and *Fusarium oxysporum*. A growth-kinetics study revealed the maximum growth of the bacterium is at the 66th hour. The SDS degrading capability was studied using Stains-all dye, with 86% degradation observed in basal medium with 0.05% SDS by the 6th day of incubation. The SDS concentration in domestic grey water was found to be 0.04% (±0.01), with maximum degradation occurring in 48 hours. A pot study was conducted in *Solanum melongena* using bacterium-coated seeds, and the phytochemical analysis on the 45th day showed a 1.89 mg/g increase in total protein, no increase in total sugars, 1.48 mg/g of proline, 0.948 mg/g of total chlorophyll, 49.4 mg/g of total phenols, and 8.6 mg/g of flavonoids in treated plants.

A078

Antimicrobial resistance in the livestock-wildlife-plant-food nexus

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Abstract

Antimicrobial resistance (AMR) is among the top global public health threats; by 2050 it is estimated that 10 million people will die due to drug-resistant infections. Global organisations recognise the environment plays a key role in the development and transmission of AMR and are calling for increased surveillance in environmental settings and a One Health response to tackling AMR.
Close links between livestock waste, fresh produce, and peri-domestic wildlife create opportunities for the spread of AMR within and between different food production systems by various routes, especially with the rise in more integrated, biodynamic farming systems.
A One Health case study was conducted to investigate the resistome in wildlife, livestock, and horticultural farming in a shared landscape. Fresh faecal samples from rodents and pigs, muck heap, soil and water were collected on a farm in the UK. Shotgun metagenomic sequencing was used to identify bacterial taxa and detect the presence of bacteria carrying antimicrobial resistance genes (ARGs) in these diverse ecological niches.
This study has provided an insight into the diverse microbial populations, including foodborne and zoonotic pathogens, resistance mechanisms and genes circulating within this biodynamic farming environment. Our results show a high diversity and abundance of ARGs and heavy metal resistance genes across the samples, including the presence of genes related to resistance of beta-lactams, macrolides, tetracyclines, aminoglycosides and oxazolidinones. Our findings indicate that highly conserved ARGs are shared across the different ecological compartments; thus, spread to humans is possible through a variety of foodborne and environmental transmission pathways.

A079

Microbial attachment to LDPE plastic beads during passage through the wastewater network.

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Abstract

Plastic pollutants are ubiquitous, particularly microplastics (MPs) found in the environment causing major concerns. MPs enter coastal waters primarily from river loading as a result from wastewater treatment plants (WWTPs), leading to global issues. MPs enter the WWTPs from the release of particles from synthetic clothing and personal hygiene products. These WWTPs are hubs for the dissemination of microplastics in the environment, however there is a gap in the knowledge with regards to the microbial carrying capacity of plastics that pass through the WWTPs and get released into the environment. We conducted a field experiment incubating low density polyethylene beads (LDPE) in influent and effluent water, and additionally tracked free floating beads during passage in wastewater from a large municipal hospital manhole to an urban WWTP where they were subsequently recovered. By measuring flow rate and turbidity using rhodamine dye and probes, we were able to determine the duration of incubation and flow from each point of the WWTP. Using flow cytometric true absolute cell counts and calculated protein content cell count we were able to quantify cell attachment to the LDPE beads. DNA extraction and 16S rRNA gene sequencing was used to determine bacterial community structure of plastic associated biofilms. We found that even after a short incubation time, distinct communities were present on the surface of the LDPE beads following exposure to each wastewater type.

A080

The effect of dietary xylo-oligosaccharide supplementation on the growth performance and gut health of commercial pigs from weaning to finish

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Abstract

Sustainable production of pork meat is needed to meet global demand. As a result of weaning, piglets can experience post weaning diarrhoea (PWD) which can lead to reduced weight gain, weight loss, and death. As such, PWD is responsible for economic loss to commercial producers. Prebiotics such as xylo-oligosaccharides (XOSs) have emerged as growth promoters in the commercial animal production industry which have shown to improve meat yield and confer host health benefits. XOSs have demonstrated their ability to improve intestinal health in weaned piglets via increased beneficial probiotic bacteria and improved gut architecture. A total of 216 piglets (28 days of age) were randomly allocated to three dietary groups: basal diet, basal diet containing 0.0017% XOS and basal diet containing 0.017% XOS. The study lasted 54 days. XOS improved growth performance. XOS significantly modulated microbial community structure (Yu & Clayton Dissimilarity, Bray-Curtis Dissimilarity), but not community membership (Jaccard Similarity) in the small intestine, determined by analysis of molecular variance (AMOVA). Linear discriminant analysis effect size (LEfSe) indicated that XOS supplementation stimulated lactic acid bacteria (LAB) more in the small intestine compared to the large intestine. XOS had no significant impact on villus height, crypt depth, crypt-depth ratio or number of crypt goblet cells (GCs) per mm^2, but did significantly increase the number of GCs per mm^2 in duodenal villi. In summary, dietary supplementation with XOS improves weaning pig growth and exerts health benefits across the porcine GIT by promoting the abundance of beneficial microbiota and improves GC expression.

A082

Advances in dairy cow health: exploring the potential of phytogenic feed additives for modulating host-microbe interactions for health benefits in dairy animals.

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Abstract

After the EU banned antibiotic feed additives in 2006, efforts to produce natural alternatives, especially plant-based, have grown rapidly. Plants have been utilized for their health benefits throughout history. The secondary plant metabolites (phytogenic compounds) produced have demonstrated potential to improve animal health and performance in agriculture. Yet the understanding of their mechanisms of action and impact on the rumen microbiome remains poorly understood. We sought to understand the impact these phytogenic compounds, already used in the dairy feed additive industry, have on the rumen microbiome and udder health. Initially, we will complete susceptibility testing on pure culture isolates of "core" ruminal microorganisms, determining which compounds have antimicrobial and antibiofilm effects. Then, *in vitro* fermentation studies using phytogenic supplemented ruminal fluid will be conducted as a model of the rumen environment, and 16s metataxonomic sequencing performed to assess alterations in the microbiome. Promising compounds demonstrating significant ruminal modulations will be used in an *in vivo* trial with dairy cows. The characterization of rumen and milk microbiomes will allow for the comparison of effects between phytogenics. Findings from this study will enhance our understanding of the impact of industry-fed phytogenics on the microbiome and how this relates to udder health, an essential step in providing confidence to farmers in the benefits of these products. It also provides a natural alternative to the use of antibiotics as growth enhancers, especially for areas of the world where they are not banned and are still heavily used, thus ameliorating the global antimicrobial resistance problem.

A083

The role of early life feeding in antibiotic resistance acquisition in dairy stock.

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Abstract

Colostrum is a vital first feed for calves in early life as it provides immunity to dairy calves, which are at an increased risk of disease and mortality due to their naïve immune system. Dairy calf loss at 3 months old falls between 6% to 12% in the UK and this risk is heightened by the antimicrobial resistance (AMR), challenge, a public health threat. Furthermore, AMR genes have been identified in calves in early life. However, the origins of AMR in calves and the role of early colostrum feeding on AMR diversity and burden is unknown. Therefore, we investigated the relationship between colostrum quality, colostrum antibiotic residue and the presence of antibiotic resistant bacteria in bovine colostrum as a potential AMR reservoir in dairy cattle. Brix refractometry and liquid chromatography mass spectrometry, were used to determine the quality and residual antibiotic residues (AR) respectively of colostrum samples (n=163) from ten farms across Northern Ireland. We also assessed the antibiotic susceptibility of bacteria colonies isolated from colostrum against six antibiotics; ampicillin, cephalexin, colistin, erythromycin, florfenicol, and ciprofloxacin following identification by matrix assisted laser desorption/ionization – time of flight (MALDI-TOF) methods. Cephalosporins (75.7%) and penicillins (18.9%) were most frequently detected in samples tested (n=90). Correlation between high quality colostrum and high AR presence was observed. Findings suggest a potential early experimental link between colostrum quality, residual antibiotic presence, and AMR. These findings provide new insights into dairy cow colostrum management, their role in AMR acquisition and the implication for One Health strategies.

A084

Biological Removal of Enrofloxacin using Microalgae and Cyanobacteria

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Abstract

Enrofloxacin is a fluoroquinolone antibiotic widely used in veterinary medicine as an antibacterial agent. It is one of the most important residual antibiotics found in the aquatic environment and has impacts on non-target organisms and ecosystem health. Microalgae are considered as a promising option for antibiotic removal due to their fast growth, strong environmental adaptability and satisfactory removal efficiency. In this study, two green algae (*Chlorella* sp. and *Scenedesmus quadricauda*) and two cyanobacteria (*Microcystis aeruginosa* and *Synechococcus* sp.) were used to assess the removal efficiency of enrofloxacin. The changes in their growth and production of extracellular polymeric substances (EPS) in response to different concentrations of enrofloxacin and antibiotic removal efficiency were studied. The results showed that enrofloxacin-treated groups had increased biomass and photosynthetic pigments in both species of green algae with increasing culture time, while variable results were obtained with cyanobacteria. A significant increase in the photosynthetic pigments was observed in *Chlorella* sp. grown with 1 mg/L and 50 mg/L of enrofloxacin compared to other concentrations. However, enrofloxacin significantly inhibited the growth and pigments in both cyanobacteria as compared to the control ($p<0.05$). Enrofloxacin increased EPS contents in *Chlorella* sp. and not in other organisms. Among the four organisms tested, only *Chlorella* sp. showed antibiotic removal efficiency with varying concentrations of enrofloxacin, and the highest removal efficiency was observed with 5 mg/L. Thus, this study provides new insights into the understanding of the ecotoxicity of fluoroquinolone antibiotics to primary producers and biological removal of antibiotics by microalgae from aquatic environments.

A085

Evolutionary history of a novel Methanosphaerula species adapted to a natural high CO_2 subsurface environment

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Abstract

Methanogens utilize one of the most ancient autotrophic pathways on the Earth - methanogenesis - which allowed them to take advantage of the atmospheric conditions in the Archean, i.e., abundant CO_2 and lack of O_2. Natural mantle degassing in the Eger Rift area (CZ) results in a similar environment. Long lasting, high fluxes of CO_2 in the subsurface provide the opportunity to study native CO_2-driven microbial processes including methanogenesis. We enriched and isolated a novel species of Methanosphaerula from drill core samples from the Hartusov mofette area recovered by the ICDP project "Drilling the Eger Rift" carried out in 2019. While this novel *Methanosphaerula sp.* itself is already an example of methanogen adapted to high CO_2 conditions, its genomics and evolutionary history further helped identifying metabolic strategies that might have been used by methanogens during the Archean, given the similar environmental conditions. The novel *M. sp.* is a CO_2-reducing hydrogenotrophic methanogen and a near-complete genome was acquired. Phylogenetically *M. sp.* stably clusters with *Methanosphaerula palustris* and other Methanosphaerulaceae according to GTDB, based on 53 concatenated Archaeal marker genes. Ancestral metabolic reconstruction through amalgamated likelihood estimation (ALE) was performed among Methanosphaerulaceae to infer the genome-level adaptation, where multiple CO_2 fixation pathways including a reductive hexulose-phosphate pathway were shown to be nearly complete besides the archaeal Wood-Ljungdahl pathway. Altogether, our findings create a window into the processes potentially used by methanogens to adapt to the Archaean atmosphere.

A086

The impact of nitrate and organic carbon on bacterial diversity in intertidal sediments of the Seine Estuary, France.

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Abstract

Nitrate reducers play a key role in removing excess nitrate from intertidal areas using carbon as electron donor. Although the positive influence of organic carbon on nitrate reduction is established, the impact of organic carbon quality on the overall bacterial diversity, community structure and activity in intertidal areas remains underexplored. This study investigates the effects of different organic carbon sources on bacterial diversity and nitrate reduction in sediments of the Seine estuary (France). Flow-through reactors containing sediments were exposed to nitrate alone or nitrate with acetate and microphytobenthos over a two-week period. We used synthetic long-read sequencing to obtain complete 16S rRNA gene sequences for comprehensive bacterial diversity characterization. Nitrate reduction rates increased eight- and three-fold with the addition of acetate and microphytobenthos, respectively. While the addition of organic carbon had little impact on bacterial alpha diversity, it changed the bacterial community structure. *Proteobacteria* and *Bacteroidota* were the dominant phyla in the initial bacterial community. The addition of both organic carbon sources led to an increase in *Proteobacteria*, possibly reflecting a selection of nitrate reducers, and a decrease in *Bacteroidota*. The functional predictions indicated an increase in the counts of denitrification and DNRA gene families with the addition of both acetate and microphytobenthos. Our results show that organic carbon availability limited nitrate reduction in these sediments and highlight different effects of acetate and microphytobenthos on bacterial diversity and community structure of the Seine estuary sediments.

A087

Validation and Quantification of CrAssphage Microbial Source Tracking (MST) Markers Targeting Human-specific Faecal Contamination in the Inflow Rivers of Taihu Lake

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Abstract

Human-specific faecal contamination has been affecting surface water and is a threat to both the environment and public health due to its potential co-occurrence with pathogens. Extended studies were conducted to detect and quantify faecal contamination using microbial source tracking (MST) markers targeting bacteria and viruses. CrAssphage, a *Bacteroides* phage discovered in 2014, showed superior human specificity and high abundance in untreated sewage water. This study validated the crAssphage markers, CPQ056 and CPQ064, for their host sensitivity and specificity in the geographical area of the Taihu watershed in China. Sewage samples and animal faecal samples collected around the watershed were used for the evaluation. In addition, surface water samples were collected from ten inflow rivers of Taihu Lake in the summer and winter, 2020. The validated crAssphage markers and previously validated *Bacteroidales* marker HF183 Taqman were quantified in the DNA samples extracted from the inflow river water. The sensitivity for sewage samples and specificity for animal faecal samples were 100%/100% for CPQ056 and 100%/96.7% for CPQ064, respectively. The crAssphage markers showed equal sensitivity and higher specificity, as compared with the *Bacteroidales* markers BacHum (100%/80.4%) and HF183 Taqman (100%/80.4%). Moreover, independent quantification of crAssphage MST markers and HF183 Taqman markers showed perfect correlations (CPQ056, r=0.9667; CPQ064, r=0.9994), indicating the practical reliability of the novel markers. CPQ056 and CPQ064 could be applied, in combination with animal-specific *Bacteroidales* markers, to detect and profile faecal contamination in sewer overflow, sewage system leakage, stormwater, and farmland drainage or be applied in the food industry.

A090

Microbial Phosphorus Cycling in Hydrothermal Vents

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Abstract

Phosphorus is one of the fundamental building blocks of life. Over the last 3.5 billion years microbial communities have evolved methods of sourcing it from various chemical compounds in the environment. The abundance of these compounds at hydrothermal vents has the potential to be quite different to background seawater due to the influence of water:rock reactions which occur deep underground. Phosphite in particular is one of the most soluble and reactive phosphorus compounds. Therefore, previous hypotheses postulate that it has been produced in serpentinizing vents where it has fuelled the growth of local microbial communities from early Earth up to the modern day. In this study, we test this hypothesis by evaluating the genomic hallmarks of phosphorus use in metagenomes and metatranscriptomes from a range of hydrothermal vents exhibiting serpentinizing and non-serpentinizing activity. Surprisingly, we find little to no evidence of bioavailable phosphite in the serpentinizing environments of Lost City, Von Damm or the Mariana Forearc. We hypothesize that this could be due to the absence of serpentinization-produced phosphite in fluids which sustain the microbial vent fauna, based on new thermodynamic models which predict little phosphite production in areas with water:rock ratios above 0.2 and temperatures below ca. 250 °C. In contrast, non-serpentinizing vents at Axial seamount exhibit relatively more potential for microbial phosphite use, potentially due to the influence of background seawater. Based on these findings, it seems unlikely that phosphite from serpentinizing vents would have fuelled substantial primary productivity in past geological eras.

A091

Utilisations of novel culture based approaches in the bioprospecting of extreme habitats for novel antimicrobial agents.

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Abstract

Actinomycetes have historically provided mankind with secondary metabolites that have led to the development of the majority of our antimicrobial agents, with Rifampicin and Streptomycin to name a few. The increased requirement for the discovery of new therapeutic and commercially significant antimicrobial agents has stimulated the need for the selective isolation of novel actinomycetes. In the present study, bacteria were isolated from 9 different soil sites of the Great Salt Plains in Oklahoma (an extreme environment) using a modified dispersion and differential centrifugation (DDC) method and plated onto media containing 0%, 3% and 10% NaCl at pH7. Selected isolates were subjected to bioactive screening against 6 pathogens *Bacillus cereus* 7464, *Staphylococcus aureus* 20231, Methicillin resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* 30104, *Pseudomonas aeruginosa*, and *Escherichia coli*. K12 at varying pH and salt concentrations. Results showed distinct zones of inhibition, across all pH and salinity levels with antimicrobial activity of the isolates recorded most commonly against Gram-positive pathogens, particularly *S. aureus* and MRSA, with antimicrobial activity against Gram-negative pathogens particularly against *P. aeruginosa* however this was more sporadic. The isolates producing the most antimicrobial activity were subjected to 16S rRNA gene sequencing to ascertain their identity and novelty within the taxonomic family, *Actinomycetales.*

A092

Efficiency of a mineral-clay association solution on the absorption/adsorption and degradation of mycotoxins in animal feeding

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Abstract

Mycotoxins, toxic metabolites produced by fungi, constitute a growing challenge for animal nutrition. These fungal contaminants proliferate in several cereals such as corn or wheat., as well as in forages. Their presence in animal feeding raises major concerns due to their harmful effects on animal performance and health and, consequently, on the safety of by-products (such as meat, milk, and eggs) intended for human consumption.

The management of mycotoxins in animal nutrition requires an integrative approach, including appropriate agricultural practices to minimize contamination, appropriate storage methods to prevent fungal growth, and the use of adsorbents and specific nutritional strategies to mitigate the negative effects of mycotoxins in animal diets.

In this context, our project aimed to study the impact of *in vitro* incorporation at rates of 1 or 6 kg/T in animal feed of a new solution based on mineral and clay synergy, on the adsorption and capture of significant chosen mycotoxins: aflatoxin B1 (AFLA), zearalenone (ZEA), deoxynivalenol (DON), and ochratoxin A (OCHRA). Thus, the inhibitor showed 100% efficiency for AFLA and between 49.5-63.80% efficiency for ZEA. Regarding DON, the tested solution demonstrated absorption rates ranging from 46.3 to 51.3% depending on the conditions, while they ranged from 67.8 to 69.3% for OCHRA. In conclusion, the development of our new mineral-clay solution dedicated to animal nutrition emerges as an efficient strategy in the overall fight against mycotoxins, aiming to protect both the performance and health of animals.

A093

Nanoplastic Corona: An emerging platform for bacterial aggregation and growth

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Abstract

Nanoplastics (≤ 100 nm) are microplastic breakdown products with unique surface properties enabling biomolecule aggregate or 'NanoPlastic-Corona' (NPC) formation. These aggregates of nanoplastic, proteins and extracellular polysaccharides (EPS) provide an ideal condition for bacteria to grow, flourish and evolve. Polystyrene nanoplastics of 30, 50 and 100 nm were selected for NPC and aggregate formation that supported maximum bacterial growth. Among various protein sources tested, maximum production ($p \leq 0.5$) of PS-NPC and bacterial growth was supported by meat-soya extract. Addition of humic acid did not have a significant impact on aggregate formation, while 2-fold increase in aggregation was found with chitin. Percentage relative aggregation revealed 62.6% and 23.2% higher aggregation with 30nm NPC than 100nm and 50nm NPC respectively. Also, NPC formation and bacterial attachment to aggregates were recorded every 24hr for 7 days using optical and Scanning Electron Microscopy (SEM). Additionally, toxicity effect of NPCs formed were investigated over a period of 5 days by flowcytometry and viable count. Environmental strain of *Pseudomonas putida* with chromosomal insertion of *lacIq -pLpp-mCherry-KmR* for red fluorescence was used to toxicity assay. Bacterial growth peaked at 24 to 48hr on all NPC aggregates with carboxyl and amine modified PS followed by inhibitory effect at day 72-120 hr. However, there was no significant ($p \leq 0.5$) inhibitory effect for non-modified NPC and steady increase in bacterial growth was detected from 24-120 hr. Given the extent of global plastic pollution, these nano-habitats act as hot spot for evolutionary processes such as Horizontal gene transfer and spread of Antibiotic Resistance Genes.

A094

The antimicrobial activity of copper sulphate as an intestinal habitat filter affecting *Salmonella* and the pig gut microbiota

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Abstract

Colonisation of pigs by *S.* Typhimurium is a major factor for transmission to humans. During colonisation of pigs, *Salmonella* encounters a hostile environment, including the resident microbiota and antimicrobials such as copper. Copper sulphate is used as a feed additive in the pig industry since EU-wide ban on the use of antibiotics as growth promoters. The most recent pandemic *S.* Typhimurium clone acquired genomic island SGI-4 encoding copper resistance genes. Copper may act as intestinal habitat filter affecting the microbiota and providing colonisation advantage for copper-resistant *Salmonella*. Hence, we tested the hypothesis that high copper in feed affects development of pig gut microbiota and metabolome in 4–6-week-old piglets. Differences between high and low copper diet were observed for the 14 species including *Bifidobacterium*, *Escherichia*, and *Lactobacillus*. A high copper diet affected faecal concentrations of metabolites important for intestinal colonisation by *Salmonella*. Altered abundance of genes responsible for metabolism and copper homeostasis were observed in the gut metagenome of pigs fed high copper diet. 100 microbial species (~25 previously uncultured) were isolated from piglets on high and low copper diet. Copper MIC and resistance gene prevalence in microbiota revealed that decrease in abundance of microbiota tended to be species with lower numbers of copper resistance genes and low copper MIC. Using an *in vitro* pig gut model, we showed that copper resistance genes increase abundance of *Salmonella* in presence of faecal microbiota and high copper. Our data indicate significant influence of copper supplementation on piglet intestinal microbiota, their function and evolution of pathogen within intestinal niche.

A095

The bacteria-derived mutant keratinase showing promising catalytic activity for keratinous waste processing

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Abstract

Keratinases are proteolytic enzymes with diverse substrate specify, which makes them valuable tool in different industry sectors, from environmental protection (waste processing, biogas production, biodegradable packaging) to medicine (treatment of acne, psoriasis). Despite the wide range of biotechnological applications, catalytic efficiency of the native keratinase is still need to be improved for utilizing it in the large-scale usage.

The current study focuses on improvement of the wild-type keratinase catalytic activity using the protein engineering strategy.

The plasmid pET42.kerA with the inserted kerA gene encoding Bacillus licheniformis BIM B-400 keratinase was created by overlap extension PCR. The site-directed mutagenesis of the target gene was performed to enhance enzyme-substrate affinity. The mutant keratinase-coding gene was expressed in Escherichia coli BL21 (DE3). The protease non-specific and keratinolytic activity of the mutant forms was estimated in 1% of skim milk, keratin azure and chopped chicken feathers degradation assay. One unit of activity was defined as the amount of enzyme required to increase the OD595 value by 0.01 per hour. The degree of hydrolysis was defined as the difference in feather mass before and after enzymatic treatment. The E. coli strain expressing mutant keratinase (A48V, E57K, N198Y) showed the highest catalytic activity on keratin azure (7,6-fold increase compared to the wild-type enzyme). The feather hydrolysis efficiency was 4,5 times higher for cell-free liquid containing the triple-mutant keratinase than for wild-type enzyme. The obtained mutant form showed promising catalytic activity enhancement for it further application in the cell-free system for processing keratinous wastes in value-added products.

A096

Characterising marine anti-fouling-associated biofilm communities.

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Abstract

Antifouling coatings are crucial for preventing biofouling on submerged surfaces, yet their influence on the microbiome remains lightly understood. The use of biocides within antifouling technology has the potential to harbour resistant communities, acting as potential reservoirs of resistance within the marine environment.

This study investigates the impact of biocidal and non-biocidal marine antifouling coatings on the composition and functionality of associated biofilms using 16S rRNA gene sequencing. This is complemented by exploratory shotgun sequencing which was used to identify resistance genes present within the antifouling treatments.

This study finds differences between the biocidal and non biocidal treatments, with unique OTUS found in each treatment. Although there is high level of species overlap between treatments, there are some diversity distinctions between treatments. Predicted functional analysis also demonstrated distinct differences in their antimicrobial resistance potential between biocidal and non biocidal treatments. By identifying AMR genes, we can address concerns related to the development and spread of antibiotic resistance within these ecosystems.

The findings from this study will not only contribute to our understanding of the ecological consequences of antifouling strategies but also offer insights into the potential implications for public health through the surveillance of AMR genes in marine environments.

A097

Comparative genomics of multi-drug resistant *Klebsiella pneumoniae* complex from clinics, wastewaters, and surface waters in the Czech Republic

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Abstract

Klebsiella pneumoniae complex (KLPNc) are opportunistic pathogens associated with a wide range of community as well as hospital acquired infection. KLPNc pose a variety of antibiotic resistance and virulence mechanisms making them a major concern for public health worldwide. The aim of this study was to compare multi-drug resistant KLPNc of hospital and wastewaters origin and reveal its potential to spread via wastewaters and contaminate receiving surface waters.

A total of 374 KLPNc strains resistant to third-generation cephalosporins or meropenem were obtained from patients suffering from urinary tract infections (UTI; n=131), hospital sewage (n=95), inflow (n=55) and outflow (n=63) of municipal wastewater treatment plants (mWWTP), river upstream (n=13) and downstream (n=17) of mWWTP in three cities in the Czech Republic. Strains were charactered using phenotyping of antimicrobial susceptibility and short-read whole-genome sequencing.

Multi-drug resistant KLPNc strains were detected in all water sources. Majority of them (95%) were extended-spectrum beta-lactamases producers. Only 15% of the isolates showed reduced susceptibility to carbapenems which was mainly associated with hospital-related origin. *K. pneumoniae sensu stricto* and *K. pneumoniae* subsp. *ozaenae* were mostly observed in isolates of UTI origin while most isolates obtained from hospital wastewaters belonged to *K. quasipneumoniae* suggesting its different source than UTI. Out of 78 different STs, phylogenetically related isolates of different source were detected only among ST307 lineage.

The study highlights the influence of urban wastewaters in the spread of highly risk multi-drug resistant clones to receiving environment.

A098

Mining the Plastisphere: Seawater biofilms as a rich source of enzymes for plastic waste degradation

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Abstract

Awareness of synthetic plastics as pollutants is widespread but alternatives to traditional recycling – insufficient to the scale of the problem – remain elusive. One attractive alternative is the biocatalytic depolymerisation of plastics into monomers that can be used as feedstock for the next round of polymer synthesis (**1**), but sourcing enzymes able to break down common plastics remains difficult.

A large fraction of plastic pollution ultimately enters the sea; seawater microbial communities, therefore, are of great relevance in investigating solutions to plastic pollution and are a diverse and under-investigated resource for biotechnology. We proposed that by investigating plastic-associated biofilms (the plastisphere) in marine systems, we can mine the latent potential within these biofilms to discover enzymes of importance for plastic biodegradation.

To this end, we generated plastisphere biofilms by incubating four plastic types in seawater and extracted metagenomic DNA from replicates over a 16-week time series. 16S amplicon sequencing was first conducted to assess community changes over the lifetime of the experiment, which informed our selection of a representative set of plastisphere samples for whole metagenome shotgun sequencing. We mined the resulting assemblies for hydrolases and oxidoreductases enriched in treatments vs controls and selected 22 for synthesis, of which 13 were soluble. We then characterised these against model substrates and a range of plastics via agarose clearance assays, spectrophotometric plate assays, HPLC product analysis assays, and FTIR.

1 – Wei, R., & Zimmerman, W., Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate. *Microbial Biotechnology* (2017) 10(6), 1302–1307.

A099

Seasonal differences in Arctic tundra microbiome activity as revealed by metatranscriptomics

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Abstract

Biota in High-Arctic tundra soils experience extreme seasonal differences in environmental conditions. During summer, topsoil communities are exposed to higher temperatures and light availability, leading to increased metabolic activity, including photosynthesis. During autumn, the onset of snow cover triggers dormancy in these communities. Nevertheless, methane emission has been shown to spike in dry soils during the cold autumn season. Seasonal changes in ecosystem functions in tundra soils are, however, still poorly understood. Here we studied gene expression in surface and subsoil microbial communities during winter, spring and summer in dry and wet tundra soils (Kongsfjorden, Svalbard) using metatranscriptomic sequencing of 85 samples. Samples taken in winter showed higher relative abundances of transcripts for posttranslational modifications including protein folding, and carbon fixation, both of which might be related to community preparation for winter dormancy for the start of the new growing season. Transcripts for methane and nitrogen metabolism had the highest relative abundances during winter, but pronounced differences between dry and wet tundra were observed, with highest transcription levels in the dry subsoil communities. In summer, transcript abundances for photosynthesis were higher in wet topsoil which is related to the presence of cyanobacteria and moss cover. We conclude that strong seasonal patterns exist in major cellular and metabolic processes, suggesting mechanisms of microbial physiological acclimation, although these differences were less pronounced between winter and early spring. A better understanding of seasonal changes in microbial response, especially during winter, is crucial in understanding the effect of global climate change on tundra ecosystems.

A100

Engineered bacterial companions to reprogramme naïve stem cells

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Abstract

Engineered living materials (ELMs), formed from synthetic consortia of living organisms and supporting scaffolds, have the potential to revolutionize multiple fields, including food manufacturing and biomedical research. However, problems remain with regards to scalability and application of the materials. The Printed Symbiotic Living Tissues (PRISM-LT) project aims to overcome these challenges, developing a system that allows for spatial control of tissue differentiation in a 3D-printed microenvironment.

One of the aims of the project is developing a platform for 3D printed bone-mimicking organoids, requiring precise control of mesenchymal stem cell (MSC) differentiation towards marrow-like adipocytes and cortex-like osteocytes. To this end, we are developing helper *E. coli* to respond to secreted metabolites from MSCs, and secrete appropriate growth factors in turn, for incorporation into the 3D printed ELM.

The first challenges we sought to tackle are the identification of promoter elements that respond to stem cells in a lineage specific manner, as well as developing slow-growing cells that do not outcompete the developing MSCs. For the former, we used spent tissue culture media from pre-adipocytes and pre-osteocytes to screen a library of *E. coli* promoters. From the ~1300 promoters screened, we have identified 10 candidates for further testing which are specifically osteoresponsive or adiporesponsive. To generate growth-deficient cells, we have deleted glutamine synthase and dipeptide permease, engineering cells that are dependent on MSC secreted glutamine from GlutaMAX breakdown for growth.

With the future incorporation of growth-factor secretion machinery, these cells will be critical to the development of a dynamic, developing biomaterial.

A102

Serial passaging to study the adaptation of plant-beneficial pseudomonads to insects

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Abstract

Plant-beneficial pseudomonads are promising candidates for the biological control of plant diseases and insect pests. Particularly, *Pseudomonas protegens* bacteria are efficient root and insect colonizer with antifungal as well as insecticidal activities. This versatility in lifestyles makes them highly interesting to study. Although many traits enabling root colonization and insect pathogenicity are already known, it is not clearly understood how these bacteria are adapted to a life in insects. We performed a serial passaging experiment with *P. protegens* CHA0 based on serial infection cycles of larvae of the crop pest *Plutella xylostella.* Although a few populations displayed an altered insect killing speed after multiple infection cycles compared to the original strain, bacterial virulence did in general not substantially change, indicating that *P. protegens* CHA0 is already well adapted to this insect species. *In vitro* screens of the passaged populations showed changes in growth rate and antimicrobial activities whereas genotyping revealed mutations in genes which are connected to the bacterial membrane structure. However, the adaptational phenotype of the identified genetic variations needs yet to be determined. Our serial passaging experiment provides new knowledge on the adaption of plant-beneficial pseudomonads to insects which is also important for their application in biological pest control.

A103

Understanding lignocellulose recognition and degradation by anaerobic gut fungi

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Abstract

Anaerobic gut fungi (AGF) are powerful degraders of lignocellulose, but their complex lifecycle is still yet to be fully resolved. Whilst AGF have been observed to be amongst the primary colonisers of lignocellulose in the rumen and preferentially recognise damaged plant tissues, it is still unknown what specific components of the lignocellulose are recognised by AGF – an important requirement for AGF to be exploited in lignocellulose bioprocessing.

AGF recognition of different sugars and their polymers will be assessed by the chemotaxis response of their zoospores. The threshold and optimum concentrations of this response will allow comparison between different components of lignocellulose and AGF species, to see if AGF zoospores' recognition of lignocellulose components are shared, or if their zoospores are primed for different sugars.

The sugars that provoke a chemotaxis response will then be assessed to see if they can support AGF growth, and the polysaccharides these sugars can be derived from will also be assessed if they can support fungal growth and/or induce enzyme expression. For polysaccharides expected to induce enzyme expression but cannot support fungal growth we will investigate how enzyme induction will be affected by the combination of carbohydrates that can support fungal growth, (e.g., glucose, cellobiose, and cellulose).

Together, these studies will provide insight into the lifecycle of AGF and elucidate how enzyme production is affected by lignocellulose-derived inducers. This knowledge will be exploited to create combinations of anaerobic fungi and lignocellulose with increased potential in selective lignocellulose pre-treatments for bioprocessing.

A104

The role of autolysins in the *Staphylococcus aureus* USA300 response to copper

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Abstract

Copper is utilized by the host innate immune system as an antimicrobial against bacterial pathogens. Many bacteria have evolved copper tolerance systems to prevent the intracellular accumulation of copper and thus reduce its toxicity. USA300 is a clone of community-acquired methicillin-resistant *Staphylococcus aureus* with heightened infectivity, but the mechanisms underpinning this are unclear. All *Staphylococci* possess the *copAZ* operon encoding a copper-efflux pump (CopA) and a copper-binding metallochaperone (CopZ). USA300 has acquired an additional operon called *copXL* encoding a copper-efflux pump (CopX) and a copper-binding surface lipoprotein (CopL), conferring copper hyperresistance. Previously, our group and others have shown these core and additional copper tolerance genes are important for USA300 survival in macrophages in addition to murine respiratory and skin models of infection.

At respiratory and skin sites, *S. aureus* also encounters different oxygen concentrations. Our studies have determined the copper responsive transcriptome in microaerophilic and anaerobic conditions. In microaerophilic conditions there is an increase in copper homeostasis mechanisms, induction of autolysis, and a shift to fermentative metabolism. Our anaerobic data show an increase in copper homeostasis and decrease in several predicted autolysins but the effect of copper on *S. aureus* autolysis in differing oxygen conditions is unknown.

We have constructed mutants in key autolysis genes downregulated in response to copper under anaerobic conditions. Phenotypic analysis of the mutants includes growth, autolysis and transcriptional response in different copper and oxygen concentrations. Together the data will establish *S. aureus* copper toxicity mechanisms in different oxygen concentrations reflective of varying host infection sites.

A105

Natural and sustainable antimicrobial fabrics for use within wound care applications.

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Abstract

Surgical site, soft tissue and wound infections are some of the most prominent causes of healthcare associated infections. The development of antimicrobial textiles and wound dressings is one method of reducing the transmission of bacterial pathogens in healthcare environments, whilst assisting the healing process and promoting localised antisepsis. This study aimed to determine the antimicrobial efficacy of a series of natural barkcloths derived exclusively from the *Ficus natalensis* and related tree species, which have been produced for hundreds of years using traditional techniques in Uganda without lasting detrimental impact to the tree. This fabric possesses many ideal properties associated with wound dressing technology, including good gaseous transmission, biocompatibility, mechanical protection, biodegradable and cost-effectiveness. Antimicrobial susceptibility and time-kill kinetic assays demonstrated that barkcloth derivatives inhibited the growth of multiple clinically-relevant methicillin-resistant *Staphylococcus aureus* (MRSA) strains and acted as bactericidal fabrics. One lead fabric demonstrated significant anti-biofilm activity against MRSA and Scanning Electron Microscopy was used to reveal morphological changes in the MRSA bacterial cell ultrastructure when exposed to different barkcloth derivatives. The observed antimicrobial properties, combined with the physical characteristics elicited by barkcloth, suggest these fabrics are ideally suited for wound and other skin care applications. This is the first example where whole barkcloth products made by traditional methods have been employed as antimicrobial fabrics against MRSA. Barkcloth is a highly sustainable and renewable product and this study presents a major advance in the search for natural fabrics which could be deployed for healthcare applications.

A108

Environmental and Genetic Factors Controlling Biofilm Growth in the Domain Archaea

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Abstract

Biofilms are multicellular structures of a complex nature which are found in diverse environments worldwide. Compared to their bacterial counterparts, little is known about the formation and dispersal of archaeal biofilms, alongside the advantages biofilms pose for archaea. To increase our understanding of archaeal biofilms, specifically their responses to environmental changes, the model organism *Haloferax volcanii* was used to test a multitude of conditions and their effects on archaeal biofilm formation and viability, including concentration ranges of salts, metals, and antibiotics using crystal violet staining and MIC assays. Experiments on the effects of motility on biofilm growth were also undertaken using a *H. volcanii* transposon insertion mutant library. Non-motile mutants were selected on soft agar for further studies, including identification of the mutation site, and growth and biofilm assays using metals and antibiotics. Different salt concentrations were shown to have varying effects on biofilm formation. For some such as $MgSO_4$ and $MnCl_2$ at both high and low concentrations, high biofilm formations were observed; for others such as $Na_3C_6H_5O_7$, higher concentrations of salts caused biofilm formation to taper off. Varying concentrations of salts, metals, and antibiotics gives an insight into the effects of environmental conditions on biofilm formation. Mutant library experiments identified several genes that affect motility including cheF2, a chemotaxis protein and HVO_3001, an ABC transporter permease. Growth curves showed similar growth for all mutants to that of the control. Screenings focusing on motility mutants will give clearer pictures into the effects of motility on biofilm formation.

A109

The effect of air pollution on the gut microbiome of wild *Bombus terrestris*.

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Abstract

Air pollution is pervasive in our atmosphere and accumulates on bee's bodies and food stores. Yet, there is limited knowledge on how pollutants affect bees and to what extent. Bees house a defined group of beneficial core gut bacteria, which is strongly linked to bee health. Our previous studies were the first to show that a major air pollutant, black carbon (BC), changes the behaviour of host associated bacteria including human pathogens and bee gut commensals. Recently, we discovered that BC exposure disrupted the gut microbiome of important UK pollinator, the buff-tailed bumblebee (*Bombus terrestris*), reared in laboratory conditions. This work provided the first evidence that BC, a single type of pollution, disrupts the *B. terrestris* gut microbiome and highlights the importance of establishing the full impact of air pollution on the bee gut microbiome.

B. terrestris are important pollinators both commercially and in nature. Wild *B. terrestris* possess a highly variable gut microbiome with a larger percentage of non-core bacteria than their managed, indoor reared counterparts. Reinforcing the need to study gut microbiome diversity in wild bees. Wild *B. terrestris* were sampled from seven UK sites with different pollution levels. The composition of the wild bee gut microbiome was highly diverse and significantly different between sites, with taxon abundance linked to local pollution level. These results illustrate that environmental factors, such as atmospheric pollution levels, are linked to the gut microbiome diversity of wild *B. terrestris*, highlighting the importance of studying the impact of air pollution on the bee gut microbiome.

A110

Microbial Diversity in the Great Rann of Kachchh and saltpans: Unveiling the Hidden Microcosm through Metagenomic approach

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Abstract

The Great Rann of Kachchh, a vast salt desert in India, presents a unique and extreme environment that has captivated scientists and nature enthusiasts. Despite its harsh conditions, this expansive ecosystem harbors a remarkable diversity of microbial life. This study investigates the microbial diversity of the Great Rann of Kachchh and two additional salt pans (Shikarpur and Mitapur) in south Gujarat, highlighting their significance as hotspots for microbial exploration in extreme environments. The analysis of the microbial diversity of samples collected from each location, comparing diversity with and without enrichment. Statistical analysis revealed significant differences in microbial diversity between enriched and unenriched samples. The enriched Mitapur salt pan sample was dominated by the Firmicutes phylum (42.14%), while the unenriched sample was dominated by the Bacteroidetes phylum (5.5%). The enriched Shikarpur salt pan sample was also dominated by the Bacteroidetes phylum (28.83%) and displayed a significant presence of Salinibacter species. Conversely, the unenriched sample showed an abundance of the Archaea Nanoarchaeaeota phylum (9.39%). The Great Rann of Kachchh samples were dominated by the Archaea domain but differed in phylum Nanoarchaeaeota (22.08%) in enriched and Euryarchaeota (6.06 %) in unenriched samples. The results highlight the importance of enrichment techniques in capturing a more complete picture of microbial communities in extreme environments. The findings suggest the potential for discovering novel enzymes for industrial applications from these diverse microbial populations. The study also reveals the presence of microbes unable to grow in artificial media, emphasizing the importance of studying them in their natural environments.

A111

Investigating the Effects of Plant Protection Products on Complex Microbial Communities

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Abstract

Crop production utilizes the application of plant protection products (PPPs) such as herbicides, insecticides, and antibiotics to crops and soils. This targets crop pests and pathogens but also results in the exposure of non-target organisms to PPPs. Bacterial exposure to these compounds may lead to the development of antibiotic resistance in bacteria in the environment and spread of antibiotic resistance genes to clinically relevant bacteria. This project considers how PPP application may contribute to the burden of antibiotic resistance.

Three antibiotics; Streptomycin, Gentamicin, Kasugamycin, and two herbicides; Glyphosate and 2-4-D, were tested for their potential selective/co-selective effects by exposing bacterial communities to increasing concentrations of these chemicals in overnight growth experiments, which informed and were followed by 7-day evolution experiments. DNA was extracted and subjected to qPCR to calculate *intI1* (a resistance marker) prevalence. Sequencing and analysis with MetaPhlAn2 were used to determine changes in community composition and ARGs-OAP was used to determine the effects on resistance gene relative abundance.

Results to date: Lowest observed effect concentrations (LOECs) for a significant reduction in growth of a complex bacterial community for streptomycin (2mg/L), gentamicin (0.25mg/L) and kasugamycin (~50mg/L) were determined by overnight growth experiments and validated by significant selection for *intI1*. Descriptive metagenome analyses revealed community composition changes (e.g. decreases in species richness at higher concentrations) and increases resistance gene relative abundance (e.g. aminoglycosides, β-lactams, tetracyclines) at concentrations lower than usage.

Results inform the effects of different PPPs on microbial communities, contributing to understanding ABR from a “One Health” perspective.

A112

Understanding variation in E. coli phylotype and antibiotic resistance phenotypes by isolation source Talatu Usar[1], Dr Sky Redhead[1], Professor Sandra Esteves[2] and Dr Cerith Jones[1] 1 - Biological and Forensic Sciences, Faculty of Computing, Engineering and Science, University of South Wales. 2 - Sustainable Environment Research Center, University of South Wales.

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Abstract

Antimicrobial resistance (AMR) is a multifaceted problem that threatens human and animal health. An increase in prolonged hospital stays, deaths, and complications due to antibiotic resistance highlight the problem. Addressing this problem requires a "One Health Perspective" and collaboration from multidisciplinary sectors in human, animal and environmental health.

This study investigates antibiotic resistance in *E. coli* from farm animals, rivers water, and sewage treatment plants. Phylotypes were compared and the relationship between these, antibiotic-resistant phenotypes, and source of isolation were investigated. Typing of *E. coli* was achieved using the Clermont *E. coli* phylotyping PCR-based method and resistance investigated using antimicrobial disk diffusion. To understand how resistance may develop, *E. coli* contamination in river water from several locations was studied, and the potential environmental impact investigated.

Overall, *E. coli* phylotypes A, BI, B2, C, D, E, and F were isolated, and we saw variation in the phylogroup from different isolation sources. The river water was the only source with the phylotype E while phylotype B1 and B2 phylotypes were only isolated from sewage and wastewater sources. Phylotype A was found in all three sources, with pig feacal sample had the highest incidence. We saw a prevalence of multidrug resistance within our strains of *E. coli* and higher resistance in *E. coli* from sewage and river water sources, including worrying levels of colistin resistance. An extended temporal and spatial analysis of *E. coli* levels in river waters and associated antimicrobial resistance is shining light on how this contributes to the wider AMR crisis.

A114

Optimisation of rhamnolipid production by heterologous expression of rhamnolipid producing genes from marine bacteria

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Abstract

Synthetic chemical surfactants (SCSs) are used in various applications. These chemicals pass through wastewater treatment plants (WWTP), where they may be partially degraded into by-products. However, WWTPs do not effectively remove SCSs due to their molecular properties, resulting in the unmitigated release of large quantities of SCSs from WWT discharge points into the ocean, where they are toxic and non-biodegradable, polluting the marine environment and ultimately impacting human health. With growing public awareness about marine pollution imposed by SCSs, demand for eco-friendly alternatives with low toxicity, high biodegradability and better environmental compatibility have increased.

A suitable alternative is biosurfactants. These surface-active, amphipathic compounds have been discovered and extensively studied from biological sources, but one group with promising marketable outlooks are rhamnolipids. Rhamnolipids are glycolipids principally found to be produced in *Pseudomonas aeruginosa*. However, pathogenicity of this species is the major limitation to commercialisation of rhamnolipids. There is a need to discover non-pathogenic rhamnolipid-producing bacteria, with the marine environment being a highly promising source of discovery due to its vastness and varying physical and chemical conditions.

In this project, novel rhamnolipid-producing genes from marine strains will be identified using various genomic analysis techniques. The identified genes will be expressed in *Pseudomonas putida* and rhamnolipid production will be optimised by genetic engineering of the heterologous strain. Through this work, we aim to produce biosurfactants with no link to pathogenic organisms, contributing to the public acceptance of microbial biosurfactants and therefore a broader positive impact on the environment.

A116

Cardboard Waste Utilisation: Exploring the Potential to Substitute Conventional Microbial Fermentation Feedstocks with Treated Landfill-Bound Cardboard for a Greener Future.

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Abstract

This research focuses on innovative approaches to repurpose landfill-bound lignocellulosic waste for use as a feedstock for the synthesis of Polylactic Acid (PLA). The aim of this research is to investigate the parameters influencing the fermentation of lactic acid by *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11842 to provide a use for this otherwise discarded material.

Lactic acid is a versatile chemical with uses across diverse industries, including the synthesis of sustainable PLA plastics. This adaptable biopolymer holds the potential for the manufacture of eco-friendly materials, with applications spanning from 3D printed medical grade implants to sustainable food packaging. The European Commission's current "Circular Economy Action Plan" aims to achieve a 60% recycle rate for paper and cardboard packaging and the research reported herein aligns with this imperative to enhance circular sustainability practices within the EU (Directive (EU) 2018/852).

This study investigates the influence of substrate concentration, temperature, pH, and aeration on the growth of lactic acid bacteria (LAB) and the yield of lactic acid using waste paper as the carbon substrate. Pre-treatment conditions such as the use of enzymatic degradation required to prepare the cardboard-derived feedstock to optimise lactic acid yield were also studied. The optimal temperature for lactic acid yields was 30°C which conflicts with the current literature which focuses on growth rather than yields.

In conclusion, this research aims to explore the potential for sustainable waste degradation that aligns with and contributes to environmental conservation, circular economy principles and the development of eco-friendly PLA plastics for a greener future.

A117

Multi-omics Analysis of Microbial Communities in a Turtle Nesting Area on the North Pacific Coast of Costa Rica

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Abstract

Actinobacteria is a phylogenetically diverse bacterial phylum widely distributed across both terrestrial and aquatic ecosystems. These microorganisms have been proven to be an exceptional source of antimicrobial compounds. In particular, actinomycetes isolated from marine environments represent a rich source of yet untapped specialised metabolites. In this study, culture-dependent and culture-independent methods were applied to study the biological and chemical diversity of bacterial and fungal communities in sediments collected in sea turtle nesting sites on the north Pacific coast of Costa Rica. First, a 16S and 18S rRNA gene metabarcoding analysis was used to characterise microbial diversity and estimate its relative abundance. Furthermore, shotgun metagenomics on selected samples allowed for a comprehensive genetic analysis, focusing on biosynthetic genes, of actinomycetes present in the sediment samples. This information was correlated with environmental metabolomics data obtained from high-resolution mass spectrometry (HRMS) analysis of sediment extracts. Finally, by using selective isolation media, an actinomycetes strain collection was created, and their bioactivity and metabolomics profile were characterised. In summary, Differences in microorganism diversity were observed, suggesting that the constant arrival of turtles to nest plays an important role in the beach microbiome. Moreover, it was observed that sediments from this area are an interesting reservoir of bioactive metabolites. Future work will focus on studying how the microorganisms characterised in this study and their metabolites would affect turtle nesting dynamics.

A119

Tackling norovirus: Investigating disinfection using a novel polyacrylonitrile-catalyst system

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Abstract

Norovirus is a highly transmissible non-enveloped virus, and a major pathogen contributing to gastroenteritis worldwide. The use of disinfectants for infection prevention is paramount to reduce its spread, especially in hospitals and low-income settings. Here, we investigated the effects of an iron-impregnated polyacrylonitrile (PAN) catalyst system on disinfection of a model murine norovirus (MNV-1) using hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). We also assessed the development of resistance in MNV-1 upon repeated treatment with H_2O_2 as well as the ability of the PAN catalyst system to overcome this.

Catalyst stability was determined by measuring the rate of iron leaching by atomic absorption spectrosopy. Antiviriral efficacy of H_2O_2 and HOCl in the presence of the catalyst against MNV-1 was determined by the BS EN 14476:2013+A2:2019 qualitative suspension test methodology, with infectious viral titre quantified for a range of concentrations and treatment times. To assess development of resistance, MNV-1 was serially exposed to H_2O_2 and passaged for ten cycles, and susceptibility to disinfection tested after each cycle.

Presence of the PAN catalyst increased the efficacy of disinfection, with lower concentrations of H_2O_2 and HOCl required for inactivation of MNV-1. Preliminary TEM analysis showed treatments with H_2O_2 plus catalyst led to higher number of damaged virions, compared to H_2O_2 alone, while the catalyst alone had no effect on virion structure. MNV-1 also became less susceptible to H_2O_2 after repeated exposure-infection cycles, suggesting emergence of resistance. Sequencing to determine specific changes in the viral genome that lead to this resistance is ongoing.

A120

The impact of iron and lactoferrin on the infant and adult gut microbiota

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Abstract

Iron deficiency, a prevalent global micronutrient malnutrition, persists despite the fortification of staple foods with iron due to the low absorption rate of dietary iron (approximately 20%). Previous studies revealed heightened pathogenesis in the gut microbiota of iron-deficient children with high dietary iron doses, yet iron supplements remain widely used across age groups. Lactoferrin, an iron-binding protein abundant in colostrum, potentially enhances breast milk bioavailability, particularly for iron. Recently employed as an adult supplement, lactoferrin is claimed to be prebiotic, fostering beneficial bacteria. This project aimed to investigate the impact of iron and lactoferrin on infant and adult gut microbiota using in vitro models.

A preliminary 24-hour fermentation experiment indicated shifts in Lactobacillaceae and Bifidobacteriaceae resulting from the separate addition of iron or lactoferrin. However, no meaningful impact was observed when iron and lactoferrin were used together. These shifts in gut microbiota were confirmed in subsequent experiments employing more realistic fermentation durations. In adult models (n=3, 16-day fermentation), the addition of iron significantly increased Lactobacillaceae by 6.82 Log_2 fold change (L2FC), Bifidobacteriaceae by 2.66 L2FC, and Enterobacteriaceae by 2.39 L2FC, while lactoferrin treatment decreased them (-1.18, -1.67, and -2.26 L2FC orderly). On the other hand, in infant models (n=3, 6-day fermentation), only an increase in Prevotellaceae was induced by iron addition (-4.01 L2FC). Furthermore, the treatments affected microbial diversity differently in adults and infants, with lactoferrin seemingly maintaining diversity in adult models and iron demonstrating a more retaining effect in infant models.

A121

Exploring a cross-feeding interaction between anaerobic gut fungi and bacteria of the rumen

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Abstract

The rumen microbiome plays a critical role in sustainable farming as the activity of microbes during digestion impacts ruminants' performance. Therefore, there is a growing interest in understanding microbial interactions as a valuable resource to modulate feed efficiency and tackle greenhouse gas emissions. While constituting a small proportion of the rumen microbiome, anaerobic gut fungi (AGF) possess an exemplary ability to digest most of the complex carbohydrates present in plant feed. AGF are considered primary degraders of plant biomass, who also support the activity of other members of the microbiome by supplying them with nutrients and metabolites, as their cross-feeding interactions with methanogens have exemplified. Nevertheless, limited knowledge exists of any other metabolic interactions involving AGF. Recently, we discovered the first example of how a rumen bacterium can support growth of AGF via cross-feeding, where the AGF act as consumers instead of primary degraders. Here, we aim to elucidate the candidate metabolites involved in this interaction, and the metabolic pathways affected. We will perform untargeted metabolomics using zwitterionic-phase hydrophilic interaction chromatography (HILIC-Z) coupled to drift tube ion mobility-quadrupole time-of-flight (DTIMqTOF) mass spectrometry to identify metabolite candidates for tracer studies. Heavy-labelled candidate metabolites hypothesised to be participating in this interaction will be used to detect breakdown products and thereby elucidate the involved fungal metabolic pathway. Finally, these results will be compared to gene expression studies from the bacteria and AGF. With this knowledge, we aim to enhance comprehensive understanding of fungal mechanisms of carbon digestion towards improvement of sustainable farming practices.

A122

Heavy Metal Bioremediation by Pseudomonas putida KT2440 Engineered with an Artificial Gene Circuit

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Abstract

Microbial bioremediation of mercury, a most toxic heavy metal element emitted by industrial pollutions, has become increasing popular for its low energy-consuming and environmentally friendly traits. *Pseudomonas putida* KT2440 has advantages of tremendous adaptability to diverse environments as well as various biodegradation or detoxification mechanisms towards both organic and inorganic compounds. Based on our previous work (Xue et al, 2021), using the surface-anchoring motifs ice nucleation protein (INP) to display the mercury-binding protein on the cell surface of *P. putida* KT2440, developing genetic circuits in KT2440 with the advanced technologies of synthetic biology could give these optimal chassis insights for environmental remediation. Here, we accomplished the design and optimization of a mercury bioremediation gene circuit with an efficient biocontainment system, in which Hg^{2+} removal efficiency was optimized ≥ 95% in the artificial wastewater system and the GMO strain escape rates were maintained ≤ 10^{-9} with the help of genetically redundancy strategy. We are also conducting the subsequent work of chromosomal expression, along with developing more cost-effective cell capture process to realize the technology (unpublished data). This newly devised system presents a promising remedy for alleviating heavy metal contamination in water sources, offering a sustainable and efficient solution suitable for industrial applications.

A123

Biomining Atlantic Salmon Skin Microbiome in Search of Eco-friendly Bioprotective Strains

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Abstract

The identification of novel antimicrobial compounds has become a priority with the emergence of multiresistant microbial strains. Bioprospecting is the exploration of natural sources in search of new bioactive compounds and this approach can provide solutions to the problem. In the context of MARBLES, an EU project involving 14 partners across Europe, we have speculated that commensal microorganisms isolated from marine ecosystems may have a key role in their hosts' defence against pathogens. Therefore, we have focused on bioprospecting the skin of healthy Atlantic salmon (Salmon salar) as a potential source of novel antimicrobial compounds that might be useful in sustainable aquaculture. Swabs taken from the skin of 3 healthy salmon in Donegal, Ireland, were cultured on LB and Marine Agar incubated at various temperatures (4°C, 28°C, room temperature), and resulting microbial isolates were identified by 16S rDNA sequencing. In total, we identified 302 culturable bacterial isolates belonging to 38 separate genera from 4 different phyla (Actinomycetota, Bacillota, Bacteroidota, Pseudomonadota). Deferred antagonism assays were carried out against a panel of 8 commerciallyrelevant Gram-negative fish pathogens. While no antimicrobial activity was detected using this specific approach, in order to unravel the encoded potential for novel bioactive molecule production associated with bacteria from this environmental niche, 18 bacterial isolates were further selected for genome sequencing and mining. The true potential of the healthy fish microbiota to provide a sustainable alternative to the high levels of antibiotics currently used in aquaculture has not yet been fully assessed, with further studies ongoing.

A125

Plastic Eating Yeast: Engineered yeast biofilms efficiently deliver plastic degrading enzymes to PET substrates

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Abstract

Plastic is a synthetic material that is widely used and is one of the most common single-use materials around the world. Since only 9% of plastic materials produced globally are recycled, a large number of these materials will end up in the natural environment, which can have devastating consequences. Therefore, it is essential to extensively explore alternative means of plastic disposal. PETase, a novel enzyme discovered in 2016, has prompted an expansion in the research of the potential bio-catalysation of PET materials. Numerous aspects, such as protein enhancement and protein delivery systems, have been published, but most applications primarily focus on obtaining high yields of the protein, which involves a costly and time-consuming process, without achieving full degradation of the PET material.

To overcome obstacles in producing and utilizing PETase, we plan to use Baker's Yeast, *Saccharomyces cerevisiae*, as an enhanced expression system. By incorporating *S. cerevisiae*'s biofilm formation capabilities and recombinant DNA codon optimization, we aim to enhance PET degradation. Our results indicate that *S. cerevisiae* can release active PETase into the extracellular environment, and that biofilm-forming *S. cerevisiae* strains can increase PET degradation. With this information, we plan using our system with other plastic degrading proteins to address the challenge of heterogeneous municipal waste, a significant obstacle in global recycling and make significant steps to the complete elimination of PET and other plastic materials.

A126

A longitudinal sampling study investigating the bacterial and antimicrobial gene content of veterinary practice sinks

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Abstract

Handwashing and utility sinks are a key part of the veterinary healthcare environment. While sinks in human healthcare facilities have been shown to be reservoirs for antimicrobial resistant (AMR) bacteria, including extended-spectrum β-lactamase (ESBL)-producing organisms and carbapenemase-producing Enterobacterales (CPE), less is known about the microbial content of sinks located in veterinary settings. We hypothesised that handwashing by staff handling animals and/or the disposal of animal-derived materials could impact the microbial communities found in sink drains.

The aim of this study was to determine the prevalence of bacteria and AMR gene content of sinks in veterinary practices over a 1-year sampling period, and to investigate how human activity alters sink bacterial composition and diversity. Sinks located in a toilet and animal consultation room were sampled at four veterinary hospitals and four veterinary general practices (GPs) in the UK every 3 months. Drain water samples and surface-associated material were collected and processed for culture- and non-cultured based analyses of microbial and AMR content. Thus far, culture-based analysis revealed *Pseudomonas* spp., *Elizabethkingia* spp. and *Spingobacterium* spp. as the most commonly isolated genera on antibiotic selective plates. Moreover, hospital consultation room sinks harboured more putative ESBL-producing organisms than sinks located in GP clinics.

Future work will focus on identifying the AMR gene content of sinks using PCR and metagenomic analysis and explore the risk of contamination due to the dispersal of organisms from sink drains during faucet use.

A128

What's in a name? Navigating the complexities of deciphering microbial species computationally via full-length 16S rRNA gene sequencing

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Abstract

Full-length 16S rRNA gene sequencing stands as a pivotal technique in microbial taxonomy, instrumental for species identification in environmental and applied microbiology. However, its efficacy is often compromised by the genetic intricacies of microbial communities, particularly the multiple copies of the 16S rRNA gene within bacterial genomes that contribute to intraspecific heterogeneity and obscure definitive species identification. We developed an integrated pipeline enhancing the rapid and accurate identification of bacterial species. 'Chopper' was utilised for high-quality read filtration, and 'Centrifuge', leveraging the RefSeq database, was employed for precise species assignment. Through comparative analyses with Nanopore sequencing and MALDI-TOF across a suite of bacterial isolates, we identified significant disparities in species assignment, notably when reads were evenly distributed across multiple species within a genus, indicating the limitations of full-length sequencing in such scenarios. Detailed examination of isolates with consistent identification issues revealed unusual alignment patterns and consistent sequence variations within the 16S rRNA genes, hinting at the presence of novel strains or uncharted genomic divergences. Our study advocates for the continual update and expansion of reference databases and the development of sophisticated bioinformatic pipelines to discern fine-scale intragenomic variations. The integration of metagenomic techniques with traditional 16S rRNA sequencing is posited as a vital next step for advancing species-level identification, thus enhancing microbial characterisation in diverse applications. In conclusion, this novel methodology promises to surmount the inherent limitations of conventional microbial taxonomy methods, offering a path towards the integration of 16S rRNA sequencing with comprehensive metagenomic analysis for unparalleled taxonomic accuracy.

A129

Optimisation of a *Pseudomonas aeruginosa* microbial fuel cell coupled with additive manufacturing of graphene electrodes to enhance power outputs.

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Abstract

Due to the ever-increasing concern of climate change, research into alternative renewable energy generation is a priority. Microbial fuel cells (MFCs) offer one potential avenue to be explored as a partial solution towards combating the over-reliance on fossil fuel based electricity generation. Limitations such as low power generation, expensive electrode materials, and the inability to scale up MFCs to industrially-relevant capacities have slowed MFC development. New electrode materials coupled with a more thorough understanding of the mechanisms in which exoelectrogenic bacteria mediate electron transfer have the potential to increase MFC power outputs.

A commercial polylactic acid/graphene (8 wt%) composite filament was used to additive manufacture (AM) graphene macroelectrodes (AM-G_Ms). The electrode surfaces were characterised and *Pseudomonas aeruginosa* was utilised as the exoelectrogen. The MFC was optimised using growth kinetic assays, biofilm formation, and quantification of pyocyanin (the redox shuttle) production (*via* liquid chromatography-mass spectrometry). Cell potential and bacterial viability was recorded at 0 h, 24 h, 48 h, 72 h, 96 h and 120 h, power density and current density were calculated.

Additively manufactured electrodes comprised of graphene (AM-G_Ms) were successfully applied in a *P. aeruginosa* MFC configuration and a maximum power output of 110.74 (± 14.63) µW m^{-2} was observed. The AM-G_Ms demonstrated power/current outputs similar to that of the carbon cloth electrodes (gold-standard) over 120 h and had no significant detrimental effect on *P. aeruginosa* viability. This study highlights the potential application of additive manufactured electrodes with the incorporation of nanomaterials as one approach to enhance power outputs.

A130

Exploring the CAZomes of sponge-derived Bacillota: unlocking ecophysiological adaptations for the discovery of industrial biocatalysts

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Abstract

Even though present at lower abundances in sponge microbiomes, cultivable members of the Bacillota phylum have been proven to be gifted producers of biotechnologically-relevant molecules in this holobiont, including antimicrobial substances, biosurfactants and enzymes. In particular, carbohydrate-active enzymes (CAZymes) have been scarcely studied by Bacillota representatives isolated from sponge specimens which is counterintuitive considering their well-known potential as sources of industrially-active CAZyme classes. Here, we dived into the CAZyme-encoding contents (CAZomes) of sixteen sponge-associated Bacillota strains to get insights into their ecophysiological relevance and, ultimately, as a biocatalytic reservoir. Fourteen strains belonged to ten different species of the Bacillus genus and the majority of them were isolated from the Demospongiae class. Overall, the CAZome size varied between 1.42% and 3.42%, with glycoside hydrolases and polysaccharide lyases comprising the predominant and the least detected CAZyme classes, respectively. Glycoside hydrolase and carbohydrate esterase families involved in the depolymerisation of cellulose, chitin and arabinan were relatively conserved across the sponge-derived Bacillota, which was also reflected by the presence of carbohydrate-binding modules typically associated with processive cleavage of these polysaccharide substrates. Despite the fewer gene copies of polysaccharide lyases, two families of this class were present in almost all strains, PL1 and PL9, both associated with pectin degradation. Elements of CAZyme gene clusters (CGCs) for the catabolism of these carbohydrates were scattered throughout the sponge-associated Bacillota genomes. Our results suggest that the poriferan Bacillota consortium might contribute actively to metabolic functions in this holobiont and constitute a valuable resource for the biodiscovery of industrial biocatalysts.

BLOCK A

Session : Exploring the skin microbiome in health and disease

A132

Topical Corticosteroids, Eczema and Staphylococci

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Abstract

The inflammatory skin condition atopic dermatitis affects around 1 in 10 children in the UK and has a strong correlation with the presence of the bacterium *Staphylococcus aureus*. Typical treatment regimens include the use of topical corticosteroids which are complimented with topical antibiotics when the lesions become visibly infected, which aids the recovery of the microbiome. Previous studies have implicated corticosteroids as anti-biofilm agents. To understand the interaction between the microbiome and corticosteroids a library containing *S. aureus* 8325-4 and NCTC6571 and *S. epidermidis* ATCC11047 strains have been evolved against 0.25% (w/v) hydrocortisone, 0.05% (w/v) betamethasone valerate, and 0.1% (w/v) betamethasone dipropionate and is currently being sequenced to determine any genomic impact from the application of topical corticosteroids. In addition, studies are being conducted to determine if topical corticosteroids have any impact on growth kinetics and mutation frequency in the presence and absence of antibiotics including fusidic acid, neomycin and rifampicin. The next steps include assessing how topical corticosteroids influence adhesion, internalisation and competition between *S. aureus* and *S. epidermidis* within a keratinocyte co-culture system.

A133

Impact of Maternal Pre-Pregnancy BMI and Mode of Delivery on the Newborn Skin Microbiome

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Abstract

Background: The maternal microbiome plays a formative role in the colonization and development of the infant microbiome through delivery. Obese pregnant women typically have higher vaginal microbiome diversity, with potentially pathogenic bacteria, that can be transferred to their newborn. Exposure to or the absence of certain bacteria during delivery can impact future susceptibility to disease, allergies, or autoimmunity.

Methods: A skin swab was collected following delivery of 39 newborns from 13 healthy weight (BMI 18.5-24.99), 11 overweight (BMI 25.0-29.99), and 15 obese (BMI ≥30.0) pregnant participants. Bacterial genera were identified via 16S rRNA amplicon sequencing.

Results: The relative abundance of *Peptoniphilus* was higher in newborns born to obese participants compared to healthy weight participants (p=0.007). The skin microbiome alpha diversity for vaginally delivered newborns was higher than Cesarean section (C-section) delivered newborns (p=0.002). The relative abundance of *Burkholderia-Caballeronia-Paraburkholderia* (p=0.038) and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* (p=0.03) was higher in C-section deliveries, while the relative abundance of *Prevotella* (p=0.039) was higher in vaginal deliveries.

Discussion: *Peptoniphilus* has been associated with skin infections, chorioamnionitis, and neonatal sepsis. With a higher relative abundance found in newborns from obese participants, they could be at an increased risk of infection. Vaginally delivered newborns had a more diverse skin microbiome consistent with vaginal flora, while the flora from C-section delivered newborns was more consistent with environmental bacteria. *Burkholdera-Caballeronia-Paraburkholderia* has been positively associated with IgE levels and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* is a dominant skin genus in plaque psoriasis patients, so their prevalence may be associated with the development of atopy or autoimmune disease.

A134

Developing a platform for real-time monitoring of Staphylococcal competition

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Abstract

The skin is our largest organ containing wide ranging environments. These contain areas that are rich and depleted of oxygen, meanwhile the nutrients are ranging and can include sources such as lipids from the sebaceous glands. The skin microbiome is also variable depending on disease states. One such example is in atopic dermatitis where *Staphylococcus aureus* dominates whereas in healthy individual's coagulase negative Staphylococcal (CoNS) species are more predominant. To understand the dynamics and interaction between CoNS species and *S. aureus* we have been developing a platform for competition assays utilising selective antibiotic markers however this platform has the disadvantage that it cannot be measured in real-time, and it is difficult to perform multiple measurements on the same system without disruption. Therefore, to better model competition between the CoNS species and *S. aureus* we are working to transform a library of consistently expressed fluorescence plasmids into *S. aureus* and *S. epidermidis* and other CoNS species to generate complimentary fluorescently tagged strains to monitor the dynamics between the species in real-time under a range of conditions. At present the genetics of *S. aureus* have been studied extensively and molecular resources are available. However, transformation of coagulase negative staphylococci has proved to be significant barrier to both us and others. This ongoing project aims to compare the use of fixed time point experiments which utilise antibiotic markers and real time assays using fluorescent markers.

A135

Analysing the bacterial induced host secretome using an *ex-vivo* human skin model

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Abstract

Studying host-bacterial interactions on the skin is essential in understanding the role of the skin microbiome since microorganisms play essential roles in maintaining skin health, preventing pathogen colonization, and influencing the immune system.

Using our established human *ex-vivo* skin models (Norwich Skin Platform) we have analysed the human skin secretome in response to bacteria. The secretome is a diverse group of proteins and other molecules released into the extracellular environment where they function in cell communication, signalling and host immune responses.

Full-thickness human skin explants were exposed to topical *S. epidermidis* (air-liquid interface) for 24 hours as a model for initial stages of infection. Conditioned media surrounding the explants was collected and subjected to protein array analysis of 105 inflammatory-associated proteins. ELISA validation was carried out on samples from parallel experiments using tissue from different skin donors.

24 proteins were regulated following bacterial exposure. Some of the most induced proteins were members of the MIP family including CCL3 and CCL4, chemokines involved in initiating immune responses. The observed induction of these proteins was validated in 3 additional donor tissue samples. We are currently exploring the cellular origin of these chemokines within the skin explants as well as mechanisms underpinning the up-regulation observed.

Analysing the skin explant secretome can help us understand the roles of bacterial exposure in skin homeostasis and infection in health and disease. We are now exploiting this robust ex-vivo skin explant model to investigate the impact of antiseptic resistance on the skin secretome under bacterial stimulation.

A136

Dual effects of different *Cutibacterium acnes* strains and specific impact of Uriage Thermal Water

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Abstract

The ambiguous role of *Cutibacterium acnes* in acne is now well demonstrated and while acneic strains such as ribotype 4 (RT4) can provoke inflammation, other strains such as ribotype 6 (RT6) are harmless and even probably exert a protective action.

In this framework, we evaluated the impact of Uriage Thermal Water (UTW). A prior exposure of the RT4 acneic strain to physiological water (PW) and epinephrin increases lipid production by sebocytes. At the opposite UTW reduces lipogenesis induced by the RT4 strain stimulated by epinephrin thus suggesting a potential reduction of the effect of stress on acne. In addition, UTW and PW have no effect on sebocytes lipogenesis induced by the RT6 non-acneic strain. We also observed that, whereas PW promotes adhesion of the acneic strain RT4 to sebocytes, UTW inhibits this process. In addition, UTW increases the adhesion of the non-acneic strain RT6 at the surface of sebocytes. In skin, both types of *C. acnes* are living together. It was then necessary to document their relations. In mixed biofilm, the RT6 non-acneic strain is dominant compared to the RT4 acneic strain. PW and UTW did not modify this balance. A similar study is ongoing in *C. acnes*/*S. epidermidis* mixed biofilms. Considering the multiple effects of UTW, we verified the occurrence of a potential impact on the resistance of the bacteria to nine antibiotics usually employed in dermatology but no variation was observed.

Taken together these results reveal that UTW can specifically target the acneic form of *C. acnes*.

A137

The growth dynamics of Staphylococcus species isolated from atopic dermatitis patients

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Abstract

It has been established that skin microbes such as *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus hominis* (*S. hominis*) play a role in the pathophysiology of atopic dermatitis (AD). However, there is an incomplete understanding about the role and interplay of different *Staphylococcus* species in the disease development. Our study aimed to determine the growth curves of *Staphylococcus* species isolated from the skin of subjects with AD and without atopy.

In this cross-sectional study, 23 AD subjects and 22 healthy controls were recruited. Pre-moistened swabbing was conducted to isolate bacteria from a lesional and non-lesional area of each subject’s body. Growth curves from 35 *S. aureus* and 12 *S. hominis* strains were generated by measuring the optical density of each strain for 24 hours. Finally, growth curve metrics for each strain was obtained using Growthcurver.

There was an inverse relationship ($\rho = -0.723$; $p < 0.01$) between the growth rate of *S. aureus* strains and the absolute amount of growth of the *S. aureus* strains. Notably, *S. aureus* strains isolated from severe AD patients tended to have higher absolute amounts of growth and slower growth rates than strains isolated from less severe AD patients.

These findings suggest that there is a key difference in the *Staphylococcus* strains found on the skin of AD subjects and healthy controls, with AD strains growing slower but to a higher burden. Further investigation is needed to elucidate whether this could result in higher levels of *Staphylococcus* metabolites that could drive the more severe manifestations of disease.

A139

The protective potential of Lab4 on challenged immortalised Human skin cells

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Abstract

The complex relationship between the gut microbiome and skin integrity, often referred to as the gut-skin axis, has garnered increasing scientific interest. This study explores the potential role of probiotic supplementation in enhancing skin health and function. Building upon previous research that links probiotics with improved skin barrier integrity, wound healing capabilities, anti-aging properties, and anti-inflammatory effects, particularly in conditions like atopic dermatitis, this *in vitro* study specifically examines the impact of the Lab4 probiotic consortia on skin cell health.

Utilising immortalised HaCaT keratinocytes under serum deprived conditions, the study aims to assess the influence of probiotic metabolites in cell free supernatants on cell viability, wound healing, barrier integrity, moisture levels, and apoptotic activity. Key findings from this investigation include a notable increase in HaCaT cell viability, enhanced migration rates indicating potential wound healing improvements, strengthened barrier integrity, potential changes in moisture retention, and a reduction in apoptotic activity.

These results suggest that the Lab4 probiotic consortia exert a beneficial impact on skin health *in vitro*. The observed decrease in apoptotic activity, potentially contributing to enhanced cellular proliferation, which may translate to improved wound healing and barrier integrity.

A140

Validation of at-home skin swabbing to analyse the human skin microbiome.

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Abstract

Studies focused on the skin microbiome often rely on participants visiting a hospital, laboratory or clinical test centre for samples to be collected by a trained employee. Expansion of such studies would be easier if participants were able to take skin swabs at home, providing that the data collected on the skin microbiome was comparable to that collected in the lab. Here, we recruited 57 participants (male and female), who were trained in-lab to complete skin swab sampling of the forearm, axilla, face and scalp. Subsequent to this, participants took a skin swab at-home 24 hours later. Swabs were then stored at room temperature and returned to the lab one week later, where they were stored at -20 °C. Bacterial DNA was extracted from all swabs and sent for 16S rRNA sequencing, where the V1-V2 region of the gene was amplified. Sequencing files were analysed using QIIME2 and the resulting files were imported into R studio as a phyloseq object and analysed using the microbiome and VEGAN R packages. Samples were grouped by body-site for a direct lab-to-home comparison. This showed that there were no significant differences in alpha and beta diversity ($p > 0.05$). Compositionally, samples from the same body-site and participant did not differ significantly. Here, we have shown that accurate sampling of the skin microbiome outside a laboratory setting is possible and generates similar 16S rRNA sequencing results to when samples are collected in a laboratory setting.

A141

Sequencing the *Staphylococcus epidermidis* genome – because its worth it.

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Abstract

Staphylococcus epidermidis is a commensal organism of the skin microbiome. It has evolved diverse mechanisms in order to survive in these environments through specific adhesins, sensing pathways as well as influencing colonisation of other species. However, it is increasingly being recognised as an important nosocomial pathogen. *S. epidermidis* causes invasive infections in selected groups, immunocompromised individuals, preterm neonates and patients with indwelling medical devices. Unlike *S. aureus, S. epidermidis* lacks canonical virulence factors, with its ability to form biofilms central to cause disease. In addition, *S. epidermidis* has an open pan-genome with 20% of its genome consisting of variable genes which allows it to rapidly acquire new traits to adapt outside the skin environment, namely antibiotic resistance genes.

Here, we characterise the genome of *S. epidermidis* NCIMB 8558 through Next Generation sequencing. In-depth analysis revealed that this strain does not encode Type II Restriction Modification (RM) systems and is therefore a suitable candidate for genetic manipulation. This genomic characterisation has facilitated the construction of a small library of fluorescent transcriptional reporter plasmids with promoters from both constitutively expressed genes and promoters from adhesins responsible for biofilm formation. These reporters will be applied to advance our understanding of gene expression in both commensal and disease phenotypes of *S. epidermidis*.

A142

Utilising an *ex-vivo* human skin model to characterise host response to *Staphylococcus epidermidis*

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Abstract

Staphylococcus epidermidis is an opportunistic pathogen frequently isolated from human skin. Its dual nature - illustrated in how it promotes skin barrier health through competition with other bacteria, but also acts a causative agent in impeding wound healing through infection – make it an ideal study organism when characterising host response.

Communication between cells is key when modelling skin response to infection. Within Norwich Skin Platform (NSP) we culture human skin explants, which maintain such cell-cell interactions, to assess the response to *S. epidermidis*, allowing for analysis of biocidal products in a physiologically relevant setting. Human skin explants were exposed (24h) to topical *S. epidermidis* and cultured at the air-liquid interface to mimic *in vivo* conditions. Octenidine and Chlorhexidine were used as positive biocide controls (applied at 0.5x MIC). Steady state mRNA levels for key genes were determined by qRT-PCR, with immunohistochemical analysis of corresponding proteins.

S. epidermidis induced antimicrobial peptide (AMP) expression that was well conserved across donors, along with genes related to bridging the innate and adaptive immune response. AMPs were found to localise to the upper epidermal layers after bacterial exposure, and culturing explants with biocides appeared to weaken this host antimicrobial response.

Restoration of skin homeostasis after infection is a balance between removing pathogens whilst not impacting healthy tissue. Our model has been able to mimic this through AMP production and upregulation of other key genes. We have begun to explore host response to common biocides by using these markers, aiming to incorporate novel formulations soon.

A143

Competition for virulence-associated carbon sources within the wound microenvironment can significantly influence infection progression.

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Abstract

The skin microbiota is a diverse and rich community which plays a major role not only in body homeostasis but also in the wound management. In the recent years, the role of commensal skin bacteria in wound healing and infection progression has become increasingly apparent. However, the precise mechanisms underpinning many of these probiotic effects are yet to be fully captured and characterised. In this work, we demonstrate that the common skin commensal bacterium *Cutibacterium acnes* has the potential to limit the pathogenicity of one of the notorious opportunistic multidrug-resistant wound pathogens *Pseudomonas aeruginosa in vivo.* We show that this effect on pathogenicity is independent of any effect on growth but occurs through a significant down regulation of the Type Three Secretion System (T3SS), the primary toxin secretion system utilized by *P. aeruginosa* in eukaryotic infections. We also show a down regulation in glucose acquisition systems, a known regulator of the T3SS, suggesting that glucose availability in a wound can influence infection progression. *C. acnes* is well known as a glucose fermenting organism, and we demonstrate that topical supplemention of the wound with glucose reversed the probiotic effects of *C. acnes*. This suggests that introducing carbon source competition within the wound microenvironment may be an effective way to prevent or limit wound infection.

A145

Exploring host-bacteria interactions in aged skin using long-read sequencing

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Abstract

Our skin hosts a complex ecosystem of microorganisms crucial for health and disease. The skin microbiota composition is known to be intricately linked to the ageing process, however, the precise cause-effect relationship remains poorly understood. The present study aims to investigate the link between human skin ageing and skin microbes. We recruited healthy volunteers from two age groups: young (18-30) and old (60+). Swabs were collected from the forehead, forearm, and foot for bacterial isolation and metagenomic profiling via long-read Nanopore sequencing. In parallel, a range of age-associated biophysical parameters were evaluated at each body site. Our analysis revealed that *Staphylococcus* species dominated the foot skin microbiome in both age groups. However, *Corynebacterium* displayed a three-fold increase in abundance in the aged forehead skin. The forehead of older volunteers also displayed a lower abundance of *Cutibacterium* and *Malassesia*. The forearm site exhibited diverse bacterial profiles, with a lower prevalence of *Staphylococcus* in the older group and a higher prevalence of *Acinetobacter* and *Moraxella*. Alpha and beta diversity, as well as biophysical parameters, did not show statistically significant differences among the groups. Current studies are exploring functional differences (eg. biofilm formation, UV resistance, influence on host/bacteria) in bacterial isolates from old versus young individuals. This project is significantly advancing our understanding of the aged skin microbiome. Our findings will provide a foundation for future perspectives and applications, ranging from managing skin age to understanding the potential contribution of bacteria to poor wound healing and skin diseases in the elderly.

A146

Staphylococcus epidermidis counteracts hyperglycaemia-induced alterations in human skin fibroblasts

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Abstract

Poor wound healing is a significant complication of diabetes mellitus (DM). Disturbances in the proliferation, migration and chemokine secretion from skin fibroblasts is considered to be an underlying contributor to chronic wounds in DM. Recent evidence reports that, under healthy conditions, *S. epidermidis*, one of the most common members of the human skin microbiota, promotes wound healing. However, little is known about how *S. epidermidis* might influence wound healing in DM. The aim of this study was to investigate how *S. epidermidis* lysates affect wound healing in DM. Human skin fibroblasts (CCD1070SK) were grown in healthy and hyperglycaemic conditions, (5.5 mM or 20 mM, respectively), followed by treatment with *S. epidermidis* lysate. Proliferation (MTS), migration (scratch assay), chemokine release (ELISA) and transcriptomics (next generation RNA sequencing) were performed. When exposed to *S. epidermidis*, skin fibroblasts demonstrated a significant increase in proliferation and migration in hyperglycaemic conditions (2.1-fold and up to 10-fold, respectively, $p<0.05$). In addition, transcriptome analysis revealed an increase in cytokine-cytokine receptor interactions, particularly the induction of the IL-17 signalling pathway, when fibroblasts were exposed to lysates. The most significantly upregulated chemokine was CXCL6, when fibroblasts exposed to lysates in healthy and hyperglycaemic level of glucose. In conclusion, *S. epidermis* lysates might induce wound healing in DM by counteracting the negative effects of hyperglycaemia on fibroblasts. However, more evidence is required to fully understand the effect of S. epidermidis lysates on diabetic wounds.

A147

Non-tuberculous mycobacteria in pets

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Abstract

BACKGROUND

Non-tuberculous mycobacteria (NTM) are a group of around ca. 240 bacteria species, most of which are free-living saprophytes. Most of them are environmental opportunistic pathogens of humans and animals. Animal-to-human transmission of the NTM-disease has not yet been proven, nonetheless, single cases of suspected zoonotic infections are being reported. The aim of the study was to evaluate the distribution of mycobacteria among pets.

MATERIALS & METHODS

A total of 300 samples were collected using sterile cotton swabs from various pets (44 cats, 31 dogs, 2 rabbits, a rat, turtle, gecko, and a snail) in a year 2023. Included within this number were swabs from: mouths (*n*=79), ears (*n*=78), anuses (*n*=66), furs (*n*=37), noses (*n*=15), and other (*n*=25). Furthermore, data on animals' medical history and habits were collected. Direct swabs of each specimen were cultured on two media (NTM Elite, Middlebrook 7H11 agar). The incubation was carried out in the dark at 30°C for 6 weeks. Colonies suspected of being mycobacteria were subjected to Ziehl-Neelsen staining. Acid-fast bacilli were identified to the species level by PCR-sequencing of the *hsp65* and *rpoB* genes.

RESULTS

Swabs originating from 15% of animals (12/81, i.e. 6 dogs, 4 cats, a turtle and a gecko) were mycobacteria-positive. Of all NTM-positive animals, 17% (2/12; 1 dog and a turtle) had a history of pulmonary infection. Altogether, 51 AFB+ colonies were recovered for PCR-sequencing analysis.

CONCLUSIONS

This study is the largest of its kind to date. Further sequencing-based investigations are required to establish the species of isolated NTM.

A148

Dissecting the non-linear relationship between the Candida albicans transcriptome and translatome during the morphological transition.

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Abstract

Pathogenicity of the commensal human pathogen *Candida albicans* is strongly associated with its ability to switch between a yeast and a hyphal morphology. The latter is a prerequisite for organ invasion and ensuing systemic infection with Candida (candidiasis). The switch to the hyphal morphology is accompanied by modest gene expression changes, with a small core set of hyphal associated genes (HAG) being transcriptionally induced. In contrast, ribosome profiling and proteomic data identifies a larger number of proteins being present at higher concentrations during the transition to the hyphal morphology. This observation implies post-transcriptional control of mRNA during the yeast to hyphal morphology, but the underlying mechanisms remain unknown. We will present data that addresses the non-linearity between transcriptional and translational output on a systems-wide level.

BLOCK A

Session : EDI - Equality, Diversity and Inclusion

A157

Gender shapes the formation of review paper collaborations in microbiology

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Abstract

What determines how collaborations form? Researchers will choose collaborators based on expertise, but social factors also likely play a role. Our goal was to investigate whether gender may play a role in the formation of collaborations in microbiology. To do this, we analysed the publication data associated with multi-author review articles published between 2010 and 2022 in three leading microbiology review journals: *Nature Reviews Microbiology*, *Trends in Microbiology* and *Annual Review of Microbiology*. The key finding of this analysis was that multi-author reviews with men as lead authors had a reduced proportion of women co-authors compared to reviews with women as lead authors. Furthermore, multi-author reviews published by all men teams were common. Taking postdoctoral researchers as the average career stage contributing to reviews, we show that all men teams are more common than would be expected if teams assembled randomly with respect to gender. Why is this important? Review articles can be considered a metric of experts in the field and can increase the profile and visibility of a researcher. They also provide the opportunity to work collaboratively on an intellectual project, and the possibility to transcend some of the logistical barriers imposed on laboratory-based research. Given the existing differences in the proportions of men and women in lead author positions, this may have important consequences for the relative visibility and progression of women in microbiology, and this homophily may have negative impacts on the outputs produced in collaborations.

BLOCK A

Session : Infection Forum (Block A)

A163

Hospital Acquired Burkholderia Cepacia Complex Carrying Blandm-1 And Blandm-5 In Ventilator-associated Pneumonia Patients And Contaminated Ventilator Tubing

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Abstract

Background: Ventilator-associated pneumonia (VAP) is a challenging condition with a significant economic impact. This study aimed to investigate the antimicrobial susceptibility patterns of BCC bacteria associated with VAP in Pakistan and to identify the possible source for preventing future outbreaks.

Methods: The blood and respiratory specimens from patients diagnosed with VAP were collected. In addition, potential reservoirs such as liquid solutions used in patient care, environmental elements, and ventilators were also screened. The isolates were identified using the VITEK2 system and further confirmed by MALDI-TOF and susceptibility profiling and screening for the acquired beta-lactamase were conducted.

Results: Out of the total 134 patients with BCC-associated VAP, B. cepacia, B. multivorans and B. cenocepacia was 68.7% (n=92), 18.7% (n=25), and 12.7% (n=17). Overall, the BCC isolates showed varying susceptibility to different antibiotics: 76.9% were susceptible to chloramphenicol, 76.1% to minocycline, 69.4% to meropenem, 60.4% to ceftazidime, 51.5% to trimethoprim-sulfamethoxazole, and 50% to levofloxacin. Resistance to ceftazidime (51/92, 55.4%) and meropenem (36/92, 39.1%) was exclusively observed in B. cepacia isolates and all isolates of B. multivorans and B. cenocepacia were found to be susceptible to both beta-lactam drugs. Regarding the carbapenemases, 15 clinical isolates contained the blaNDM variants i.e., blaNDM-1 and blaNDM-5. Significantly, all 20 carbapenem-resistant isolates from the ventilator tubing were identified as B. cepacia, and they were found to harbor both the blaNDM-1 or blaNDM-5 genes.

Conclusions: Acquired beta-lactamases are alarming factors that could lead to significant outbreaks, This underscores need for comprehensive epidemiological investigations and effective outbreak control measures.

A165

In silico analysis of Vi-positive *Salmonella* Dublin genomes

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Abstract

The Vi antigen is a capsular polysaccharide, encoded by the *viaB* locus, commonly associated with *Salmonella* Typhi. It is occasionally detected in *S.* Dublin, which naturally causes typhoid-like disease in cattle. However, its contribution to pathogenesis in cattle remains unknown. The aim of this study was to perform *in silico* analysis of the genome structure and *viaB* locus of three Vi+ *S.* Dublin strains isolated from humans and cattle, whose Vi expression differed *in vitro*.

Long-read sequencing was performed on the three strains, using a 96-plex, native-ligation method on an Oxford Nanopore PromethION flow cell. Short-read sequencing was also performed from the same DNA extract on the Illumina NextSeq platform. Hybrid assemblies were made by constructing a long-read assembly and polishing with short-reads. The data showed that the entire *viaB* locus was highly conserved across the strains, however polymorphisms and a small deletion were detected in one strain that may explain its loss of Vi expression. Large-scale genome structure variation was observed along with differences in sequence types and the presence of plasmids.

Further studies will explore whether the genome structure and plasmid repertoire affects the Vi phenotype of the *S*. Dublin. Various *in vitro* models will assess the impact on Vi expression, localisation, and recognition by the bovine immune system. Alongside this, mutants of the *S.* Dublin *viaB* locus have been created, and ongoing studies will probe their impact on interactions with bovine cells and the host, including by use of novel surgical models to study invasion, inflammation, and systemic translocation.

A166

Identification and Functional Analysis of the Contribution of the avrA Gene to Vancomycin Resistance in Enterococcus faecium

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Abstract

The growing challenge of vancomycin-resistant enterococci underscores the need for a comprehensive understanding of theme mechanisms that contribute to vancomycin resistance in the important opportunistic pathogen *Enterococcus faecium*. This study builds on the previous discovery of the *avrA* gene, through Transposon sequencing (Tn-seq) library screening and RNA-seq analysis during growth of *E.faecium* in the presence of vancomycin. The *avrA* gene is speculated to be contributing to the resistance of *Enterococcus faecium* to vancomycin.

We will use a novel CRISPR-Cas12a-based methodology for genetic manipulation to generate a targeted deletion mutant in *avrA*. We will then examine the changes in vancomycin susceptibility, growth, and the expression of the *vanA* resistance genes with the *avrA* deletion mutant..

Our findings will offer new insights into the molecular foundations of vancomycin resistance in *E. faecium* and could potentially open pathways for the development of novel antimicrobial agents against multidrug-resistant Gram-positive bacteria.

A167

The longitudinal assessment of anti-virulence gene expression in Pseudomonas aeruginosa grown in cystic fibrosis sputum mimics

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Abstract

The accumulation of mucous within the pulmonary tract is a hallmark of Cystic Fibrosis (CF), and its presence contributes to increased susceptibility to chronic lung infections. *Pseudomonas aeruginosa* is commonly isolated from people with CF (pwCF), and its presence contributes to morbidity and mortality rates. *P. aeruginosa* infection can be managed with antibiotics; however, such therapeutics rarely alleviate the infection once it becomes chronic. Antibiotic resistance develops over time and therefore new therapeutics are needed. Anti-virulence therapeutics weaken a bacterium rather than kill it and represent a novel approach to treating *P. aeruginosa* infection in pwCF. However, many of the approaches used for studying traditional antimicrobials may not be applicable to anti-virulence agents. Such therapeutics require alternative approaches for evaluation. Despite their potential, the effectiveness of anti-virulence therapeutics in conditions relevant to the CF lung is poorly understood.

This work assesses the expression of various anti-virulence genes (*lasR, rhlR, lasB, pqsA, exoS, exoT, exoY, pvdL, fpvB)* in *P. aeruginosa* PAO1 and PA14 grown in Synthetic CF Media 2 (SCFM2) and CF lung media (CFLM) compared with Lysogeny broth (LB) across several time points (2h, 6h, 24h, 72h). This allows us to understand the dynamics of the *P. aeruginosa* virulome in models reflecting the CF lung. In establishing these dynamics, the impact of external influences including lung components and the polymicrobial community on virulence gene expression can be established. Development of a new generation of infection therapeutics requires detailed understanding of host environment and pathogen interactions particularly in chronic lung infections.

A168

The immunokinetic profiles of patients with Staphylococcus aureus bacteremia

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Abstract

Sepsis affects 147,000 individuals in the UK, with ~40,000 deaths/year. During sepsis, proinflammatory cytokines accumulate systemically. Rather than helping the immune system fight invading pathogens, this can trigger severe immunopathology often leading to multiple organ disfunction and death. Concurrently, the compensatory anti-inflammatory response also becomes activated during sepsis and results in inhibitory cytokines being produced in addition to expression of cell surface checkpoint receptors, which may help limit the immunopathological damage in sepsis. Yet, the expression of multiple checkpoint receptors in sepsis patients has not been widely investigated. Methods: We embarked on a preliminary analysis of immune responses on n=8 Staphylococcus aureus (SA) bacteremia sepsis patients, within the first week and at 2 weeks of hospital stay, in comparison to n=10 healthy donors. We designed multiparametric flow cytometry panels to study kinetic variations of the phenotype of antigen presenting cells (APCs) and T cells isolated from the blood of sepsis patients, focusing on the expression of multiple checkpoint receptors. Results: The APC panel demonstrated that although CD14+ CD16+ monocytes/macrophages had increased in frequency, these expressed less markers of activation (HLA-DR,CD40,CD86) and more checkpoint receptors in sepsis patients (at any time point) than healthy donors. The adaptive immunity panel demonstrated that CD4+ and CD8+ T cells expressed higher amounts of multiple checkpoint receptors by day 7 of sepsis, a trend that became more significant later, by day 14 of sepsis. Conclusion: Our study suggests immune cells from sepsis patients showed and maintained a predominantly immunosuppressive phenotype during hospital stay

A170

Carbapenem and Colistin resistant *Acinetobacter* spp. cultured from hospital sanitary ware.

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Abstract

Background: 70% of Hospital Acquired Infections (HAIs) are directly linked to Antimicrobial Resistance (AMR). Throughout 2019 in Ireland there were over 7,000 AMR associate HAIs with over 200 deaths. Our team is investigating Irish hospital sanitary ware as reservoirs for carbapenem and colistin resistant *Acinetobacter* species. In Ireland, *Acinetobacter* resistance remains low in comparison to other countries within the EU. However, these pathogens have increased by 21% from 2019.

Methods: *Acinetobacter spp.* isolates were cultured from swabs taken from hospital sanitary ware. The swabs were taken from three separate rooms, at three different time frames, and on three different dates. Each isolate was enriched on selected agar for Imipenem resistance.

Results: There were over 200 Imipenem resistant *Acinetobacter* species isolated. 55% of isolates came from the showers, 34% from the sinks, and 9% from the toilets. ASTs were performed on each isolate using the disk diffusion method according to the EUCAST/CLSI 2023 guidelines. We found 84% of isolates were resistant to Meropenem and further double-disc synergy testing with EDTA suggested the possibility of Metallo-β-lactamases (MBLs) present. A total of 107 isolates were MDR and exhibited resistance to imipenem, meropenem, and cefotaxime in addition to ceftazidime, gentamicin, and amikacin. Microbroth dilutions were used to test the isolates for Colistin resistance. From 72 isolates tested 22% had MIC values ranging from 4ug/ml - 32ug/ml, indicating Colistin resistance.

We conclude hospital sanitary ware acts as a reservoir for carbapenem resistant, colistin resistant, and MDR resistant *Acinetobacter* species

A171

Menadione: A potential antimicrobial agent for *Helicobacter pylori* infections?

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Abstract

Helicobacter pylori is a human pathogen that infects and thrives in the gastric region. It is the causative agent in many gastric and duodenal conditions such as duodenal, gastric and peptic ulcers and gastric cancer. The treatment and eradication of this organism is becoming increasingly difficult because of antibiotic resistance. Therefore, there is a rising need for alternatives to currently used antibiotics and interventions that restore the efficacy of antimicrobial agents for the treatment of *H. pylori* infections.

Menadione, an analogue of 1,4-naphthoquinone (1,4-NQ) is a synthetic vitamin K3 with pharmaceutical potential and has been reported to have antibacterial activity against many multidrug-resistant organisms. We tested menadione's antibacterial effect on 14 *H. pylori* clinical isolates with varying susceptibility to the antibiotics currently used for treating *H. pylori* infection and found the minimum inhibitory concentration to be 0.31 mM.

The synergistic effect of menadione with antibiotics (clarithromycin, metronidazole, and levofloxacin) were also studied, using selected *H. pylori* isolates that had previously been identified to be resistant to these antibiotics. For some of these isolates, exposure to 0.01 or 0.001 mM menadione increased the diameter of the zones of inhibition in metronidazole and clarithromycin disc diffusion assays. This suggests that there is potential for use of sub-MIC doses of menadione to restore susceptibility of drug resistant *H. pylori* to antibiotics, as well as potential use of higher doses of menadione as an antimicrobial agent to treat *H. pylori* infections.

A172

Using RNAseq to understand the responses of *Salmonella* serovars to bile

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Abstract

Salmonella enterica is a zoonotic pathogen of global importance causing an estimated 93 million human infections annually that are commonly acquired via the food chain from farmed animals. There are over 2500 *Salmonella* serovars that cause disease ranging from self-limiting gastroenteritis (e.g.: *S*. Typhimurium) to systemic typhoidal disease that can lead to asymptomatic carriage in organs like the gallbladder (e.g.: *S*. Dublin in cattle, *S*. Choleraesuis in pigs, *S*. Typhi in humans). Bile is an environmental cue found in both the gastrointestinal tract and gallbladder albeit at different concentrations. Whilst *S*. Typhimurium and *S*. Typhi are known to respond differently to bile, little is known about host-restricted serovars like *S*. Dublin and *S*. Choleraesuis. In this project, we used RNAseq to identify global transcriptional differences between *S*. Dublin, *S*. Choleraesuis and *S*. Typhimurium in LB broth and bile at intestinal (3%) and gallbladder concentrations (10%). We found differences in the basal expression levels of genes in the *fli*, *flg* and *che* motility operons in LB broth with *S*. Typhimurium expressing them at higher levels than *S*. Dublin and *S*. Choleraesuis. This correlated with the greater swimming motility observed for *S*. Typhimurium compared to *S*. Dublin and *S*. Choleraesuis *in vitro*. In the presence of 3% bile, motility genes were downregulated in all serovars at varying levels and reduced swimming was observed in the presence of 3% bile. Differences in the expression of SPI-1 (invasion) and SPI-2 (intracellular survival) loci between serovars have also been observed and are being investigated.

A173

In Vitro ALI Co-Culture Model Using Primary Human Airway Epithelium & Non-Autologous Neutrophils to Study Host-pathogen and Immunoglobulin Interactions

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Abstract

Polymorphonuclear neutrophils (PMN) play an important role in infectious and inflammatory diseases. They serve as first line of defense against pathogens such as viruses and bacteria. The project's aim was firstly to develop an in vitro primary cell-based co-culture model using human airway epithelium (MucilAir™) and heterologous PMNs. Secondly, to investigate the potential of immunoglobulin purified from human plasma to reduce the level and consequences of pathogen infection in the conducting airways of the respiratory tract.

Adding PMN to the apical side of the MucilAir™ did not affect tissue integrity as assessed by transepithelial electrical resistance (TEER). The cilia beating frequency was increased in the presence of PMNs and neutrophil enzymes (MMP9, MPO, NE) were released into the apical compartment of the co-culture. Visualization of PMNs collected and stained after 6h of coculture confirmed the presence of intact cells with segmented nuclei.

The apical side of the MucilAir™-PMN co-culture model was infected with Streptococcus pneumonia serotype 19F and growth was quantified 18 hours post-infection. Adding PMN alone did not influence the growth of Sp19F on airway epithelium. However, in the presence of plasma-derived IgG or IgA (500µg), and PMN a trend towards reduced growth of Sp19F was observed at low inoculum. No effect on bacterial growth was observed at the same conditions with high Sp19F inoculum.

In conclusion, we have developed the basis for a novel complex epithelial co-culture model involving immune components. This model is a promising tool to study respiratory infections and related treatments.

A174

Prolonged Subculturing of Aspergillus fumigatus on Galleria Extract Agar Results in Altered Virulence and Sensitivity to Antifungal Agents

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Abstract

Aspergillus fumigatus is an environmental saprophyte and opportunistic fungal pathogen of humans. The aim of the work presented here was to examine the effect of serially subculturing *A. fumigatus* on agar generated from *Galleria mellonella* larvae in order to characterize the alterations in the phenotypes that might occur. The passaged strains showed alterations in virulence, antifungal susceptibility, and in protein abundances that may indicate adaptation after 25 passages over 231 days on *Galleria* extract agar. Passaged strains demonstrated reduced virulence in *G. mellonella* larvae and increased tolerance to hemocyte-mediated killing, hydrogen peroxide, itraconazole, and amphotericin B. A label-free proteomic analysis of control and passaged *A. fumigatus* strains revealed a total of 3329 proteins, of which 1902 remained following filtration, and 32 proteins were statistically significant as well as differentially abundant. Proteins involved in the response to oxidative stress were altered in abundance in the passaged strain and included (S)-S-oxide reductase (+2.63-fold), developmental regulator FlbA (+2.27-fold), and histone H2A.Z (−1.82-fold). These results indicate that the prolonged subculturing of *A. fumigatus* on *Galleria* extract agar results in alterations in the susceptibility to antifungal agents and in the abundance of proteins associated with the oxidative stress response. The phenomenon may be a result of selection for survival in adverse conditions and highlight how *A. fumigatus* may adapt to tolerate the pulmonary immune response in cases of human infection.

A175

DETERMINATION OF EPIYA MOTIF OF HELICOBACTER PYLORI CAGA AND CAGE GENE CORRELATTED WITH CLINICOPATHOLOGICAL OUTCOMES IN THAI PATIENTS

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Abstract

The oncogenic potential of Helicobacter pylori cagA virulence factor is linked to its polymorphic EPIYA motifs. The study was aimed to determine cagA, cagE gene and EPIYA motifs as well as the relationship with clinicopathological outcomes. A total of 230 H. pylori DNA extracted from biopsies were confirmed to be H. pylori infection using 16s rRNA and ureC gene detection. We observed 152 H. pylori-positive patients and determined the cagA, cagE and EPIYA regions using real-time PCR and PCR-based sequencing. H. pylori-positive patients with cagA and cagE was 90% and 97%, respectively. CagA and CagE combination was 57%. Among the cagA positive isolates, East-Asian-type containing EPIYA-D was 88% in CG, 100% in AG and 87.5% in IM. Western-type containing EPIYA-C was 58% in CG, and 25% in IM but no case of EPIYA-C was detected in AG. The majority of EPIYA-A and EPIYA-B were obtained 69%-100% of CG, AG and, 87.5% and IM. The results indicated that patients infected with East-Asian type had significantly higher in AG and IM whereas western type was more common in gastritis than AG and IM. Among CagA and CagE-combination, the EPIYA-D motif was correlated with IM (p-value <0.001). The pattern of the EPIYA-D motif had common variance of 300 bp (44.8%), 400 bp (13.8%) and combination variance (11.5%). H. pylori harboring cagE, cagA and in combination with EPIYA-D motif was related with clinicopathological outcomes. The EPIYA-D motif might be a useful marker for the identification of gastritis in Thai patient.

A176

Characterising two novel *B. pertussis* virulence factors to improve our understanding of virulence and host colonisation.

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Abstract

With Pertussis incidence increasing worldwide, it is important we improve our understanding of *B. pertussis* virulence, particularly regarding host colonisation, the pre-requisite to infection and transmission. This will aid vaccine development to limit inter-host transmission and thus incidence. Virulence is regulated by a two-component sensory kinase system, BvgAS, with three distinct phases: Bvg-; Bvgi; and Bvg+. The former is considered avirulent, the latter virulent, and the intermediate Bvgi phase to be important for host colonisation and transmission. Investigating Bvg-regulated genes allows the identification of unexplored putative virulence factors. We are characterising two co-localised Bvg+/Bvgi genes with hypothesized importance in virulence/transmission.

Protein homology predictions suggest their potential role as adhesins, unsurprising given the high redundancy of *B. pertussis* adhesins and the importance of host colonisation for a respiratory pathogen. Further, high relative expression within both Bvgi and Bvg+ is characteristic of adhesins, with the expression of toxins greatly reduced in the intermediate phase. *In vitro* infection studies using knockout and complement mutant strains found reduced binding potential to lung epithelial cells in single and double knockout mutant strains, which were rescued upon complementation. Cytotoxicity of mutant strains to lung epithelial cells and to human macrophages were unaffected.

Understanding the role of these genes in *B. pertussis* virulence may facilitate a greater knowledge of host colonisation and thus also transmission, with potential implications in future vaccine development.

A178

Domestic laundering of Healthcare Textiles: a potential source of antibiotic resistance?

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Abstract

Microorganisms can survive domestic laundering on textiles, having the potential to cross-contaminate healthcare and home environments. The effect of repeated exposure to domestic detergents is not well understood. Sub-lethal exposure to antimicrobials can drive antibiotic cross-resistance.

Aim: To determine the development of bacterial resistance to domestic non-biological laundry detergents and subsequent effects on antibiotic susceptibility.

Methods: Stepwise training in broth was used to develop detergent resistance/tolerance in Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa by exposing them to sub-lethal concentrations of non-biological domestic detergents (powder and liquid). The mutants created were assessed for cross-resistance to clinically relevant antibiotics using a disc diffusion method. Whole genome sequencing of mutants demonstrating antibiotic resistance (EUCAST clinical breakpoints) was then conducted to identify any resistance-inducing genes.

Results: Repeated exposure to laundry detergents increased resistance in the bacteria, from 180µg/ml to 270µg/ml (powder) and 0.000675µl/ml to 0.60µl/ml (liquid) in S. aureus, 0.00675µl/ml to 2.05µl/ml (liquid) and 18µg/ml to 27µg/ml (powder) in P. aeruginosa, and 0.000675µl/ml to 10.36µl/ml (liquid) in K. pneumoniae. In parallel, increased resistance to antibiotics was observed in S. aureus to Moxifloxacin 5µg, Fusidic Acid 10µg, Penicillin G 1 unit, Tetracycline 30µg, Rifampicin 5µg, and Oxacillin 5µg.

Conclusion: Sub-lethal concentrations of detergent can induce resistance to domestic laundry detergents and cross-resistance to antibiotics, particularly in S. aureus. This is of particular concern in the domestic environment where washing detergent concentrations are not regulated/maintained or monitored and is reliant on accurate individual user dispensing. This could have implications for the spread of antibiotic-resistant bacteria.

A179

Investigation of longitudinal RSV mucosal antibody responses in healthy individuals

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Abstract

Respiratory syncytial virus (RSV) infection poses a considerable global health burden, especially in infants, the elderly, and immunocompromised individuals. Given limited research on RSV and the recent introduction of a vaccine, it is crucial to deepen our understanding of the pathogen and the immunity individuals elicit upon infection. RSV initially infects the upper respiratory tract, but there is limited information of the mucosal concentrations of IgA antibodies directed to RSV specific proteins long-term. Therefore, we sought to determine the longitudinal mucosal immune responses in healthy individuals.

Synthetic absorptive matrix (SAM) strips were used to isolate mucosal lining fluid (MLF) of the nasal cavity from healthy healthcare workers enrolled into the PITCH study across timepoints that covered RSV seasons during 2021-2023, with IgA antibody titres to RSV antigens determined through a multiplexed Luminex serology assay.

We were able to detect IgA antibody titres to RSV pre-fusion, post-fusion, and G-protein from both RSV A and B strains in the SAM strips. Fluctuations in IgA levels were noted from 2021 to 2023, with increases aligning with the onset of RSV seasons. Further ongoing work is being performed to understand IgA, IgM, and IgG (and subclasses) in MLF and associated serum.

These findings will significantly enhance our understanding of the immune response to RSV infection, a crucial consideration with the planned vaccine distribution in the UK. Furthermore, the SAM strips' ability to isolate mucosal antibodies enables their integration with other sample collection methods, providing a comprehensive insight into RSV infections and reinfections.

A180

The Burden of Antibiotic Resistance in Community Acquired Pneumonia (CAP)

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Abstract

Community-acquired pneumonia (CAP) represents a significant public health challenge and is a leading cause of mortality among children worldwide. This condition, often caused by bacterial infections, places a substantial burden on healthcare systems, especially in resource-limited settings. The predominant causative agent of CAP has traditionally been *Streptococcus pneumoniae*. However, recent trends indicate a shift in the microbial landscape, with an increase in cases attributed to other bacteria, notably *Staphylococcus aureus*. This shift is partly attributed to widespread pneumococcal vaccination, which, while reducing the incidence of *Streptococcus pneumoniae* infections, may be altering the ecological balance, allowing other pathogens to emerge.

Our research involves analysing a unique collection of bacterial cultures derived from Malawian children diagnosed with CAP, sampled before the administration of antibiotics. Preliminary analysis revealed a significant presence of presumptive *Staphylococcus* species. This finding is critical, given that *Staphylococcus* is notorious for its resistance to multiple antibiotics.

Our subsequent investigations focused on the antibiotic susceptibility of these isolates. We found that a substantial majority (88%) exhibited resistance to erythromycin, an antibiotic frequently used in Malawi, often due to its availability. This resistance is concerning, considering that amoxicillin, the recommended first-line treatment for paediatric pneumonia, is not always readily available in many low-income countries.

We are currently examining the genetic basis of this resistance and assessing how these bacteria evade the effects of commonly used antibiotics. Furthermore, our future studies are set to evaluate the potential of novel antimicrobial compounds as adjunct therapies with erythromycin.

A181

Importance of database selection when comparing genotypic with phenotypic antimicrobial resistance data for uropathogens: case study from Egypt

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Abstract

Introduction: Antimicrobial resistance (AMR) poses an escalating threat to public health, due to the rapid global dissemination of multidrug-resistant phenotypes. Clinical microbiology laboratories in low- and middle-income countries (LMICs) rely on antimicrobial susceptibility testing (AST) to personalize treatment and PCR to track high-risk clones. Decreases in the cost of whole-genome sequencing (WGS) make it an increasingly attractive alternative for strain characterization in LMICs, where there is a crucial need to track high-risk clones that contribute to a high burden of infectious diseases.

Methods: We evaluated the effectiveness of available databases – ResFinder, RGI/CARD, and AMRFinder – for predicting AMR marker genes in 132 diverse uropathogens recovered from catheter-associated urinary tract infections in an Egyptian hospital, comparing WGS-based and phenotypic AST data.

Results: Comparison of AST with WGS data yielded a concordance of 87%, 80% and 79% for ResFinder, CARD and AMRFinder, respectively. Discordant results were mainly due to phenotypically susceptible isolates harbouring genetic AMR determinants. *In silico* AMR prediction was more promising for antibiotics like quinolones, and *Enterobacterales* rather than *Pseudomonas* spp., which have poor concordance. Notably, the databases demonstrated a higher incidence of major errors than very major errors.

Conclusion: The study represents a first report from Egypt emphasizing the need to understand discordant cases and use curated bioinformatics databases, and the necessity for improved marker-gene annotations tailored to specific antibiotics rather than antibiotic classes. Within the realm of clinical microbiology, improved curation of *insilico* AMR predictions will ensure greater reliability between computational and phenotypic profiles of AMR.

A182

Regulatory cross-talk supports resistance to Zn intoxication in *Streptococcus*

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Abstract

Copper (Cu) and zinc (Zn) are mobilised by the host to intoxicate invading pathogenic bacteria. Defined resistance mechanisms are used by some streptococci and other bacteria to subvert intoxication by transition metals, which typically comprise efflux pumps that expel metal ions from the bacterial cell. We previously defined systems encoded by *copA* and *czcD* in Group B streptococcus, which support resistance to Cu and Zn and alter host-pathogen interactions. Using transcriptomics and transposon directed insertion site sequencing we have identified a suite of genes with novel contributions to metal ion resistance in Group B streptococcus, including those encoding cell signalling, metabolic and putative cell wall synthesis processes. Mutational analysis and growth assays reveal a remarkable interplay between metabolic processes and metal resistance phenotypes during Cu (1) or Zn intoxication (2). We also reveal cross-talk between Cu and Zn resistance systems, effected by the Cu-sensing CopY repressor (4), required for survival during Zn intoxication. These insights broaden the horizon for research into mechanisms of metal ion resistance in streptococci and beyond.

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A184

Identification of metal complexes with broad-spectrum antibacterial activity.

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Abstract

With the increasing burden of antimicrobial resistance, the need to uncover novel antibiotic agents becomes increasingly critical. This urgency of this need is enhanced by the void within antibiotic discovery, with the identification of *de novo* antibiotics declining with time. One strategy that holds promise is the potential for drug repurposing, whereby compounds originally intended for non-antimicrobial use can be repurposed for such gain. This option removes the need for *de novo* drug discovery, beneficial due to the multiple challenges surrounding this. In the present work, we have identified a set of metal complexes with *in vitro* bactericidal and bacteriostatic activity, repurposed from their originally intended use as novel chemotherapeutic agents. Multiple complexes demonstrate activity against the three *Mycobacterium abscessus* subspecies, *Mycobacterium bovis* BCG and/or ESKAPE pathogens, revealing several hit compounds. Our lead candidates demonstrate broad-spectrum inhibition of multiple antibiotic resistant pathogens, including acid-fast, Gram positive and Gram negative species. Such compounds therefore demonstrate significant promise for further development.

A185

Assembly and secretion mechanisms of the *Bacteroides* T6SS

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Abstract

Background

The biodiversity of the human gut microbiota has been shown to be linked to homeostasis and physiological regulation of distant tissues. *Bacteroides* is an abundant genus in the intestinal microbiota and contains members that use type VI secretion systems (T6SS) to actively kill competitors. These systems are divergent from their proteobacterial homologues and have unique structural subunits. We exploit such differences to study fundamental aspects of T6SS structure and function.

Methods

We performed a genetic screen of protein–protein interactions between structural genes of the T6SS from *Bacteroides fragilis* and performed biochemical and structural characterization with size exclusion chromatography, crystallography, and cryo-EM of some of the proteins that showed interactions. We then applied structural and mathematical modelling to understand the assembly pathway of one of these subcomplexes.

Results

We discovered that one major and four minor subunits of the Hcp1-like superfamily interact with each other. These proteins have very low sequence similarity but have similar structures. Our biochemical and structural studies suggest that interactions between these proteins occur hierarchically. We also provide evidence for their role in secretion.

Conclusion

Our results are consistent with an assembly model of the minor Hcp subunits in which protomers interact in a cooperative and concerted manner to mediate secretion.

A187

Biofilm formation, motility and morphology of Cronobacter sakazakii exposed to polyunsaturated fatty acids

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Abstract

Background

Cronobacter sakazakii is a food-borne pathogen and infections will be notifiable for those under 1 year as of January 2024 (FDA, 2023). Currently, inhibition methods rely on manufacture and reconstitution guidelines. Fatty acids (FA) are a possible method of control, with reports showing FA can be bactericidal and regulate virulence mechanisms related to motility and biofilm formation. This research explores the efficacy of three long chain fatty acids: α-linolenic, oleic and linoleic acid as potential antimicrobials to combat C. sakazakii.

Methods

Biofilm formation was assessed using the specific biofilm formation inhibition assay (Naves et al., 2008). C. sakazakii was exposed to each FA at 500, 250 and 125µM, for 24, 48 and 72 hours at 37°C. Resulting biofilm formation was compared to untreated controls and % biofilm inhibition determined. Motility was analysed using the soft agar assay with chromogenic Cronobacter agar. Morphology was determined using Scanning Electron Microscopy (SEM).

Results

250µM α-linolenic acid reduced biofilm formation by 90% in complex media and 38% in defined media after 24 hours. Oleic and linoleic acid also reduced biofilm formation at 250µM. Regarding motility, C. sakazakii with FA showed reduced motility when compared to the untreated control. SEM images showed no morphological changes following 12-hour exposure to each FA.

Conclusion

Long chain unsaturated FA including α-linolenic acid reduced biofilm formation and motility of C. sakazakii but did not impact morphology. This data may inform novel inhibition approaches.

A188

Untangling the microbial interactome in chronic infection

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Abstract

Although we have multiple methods to catalogue which microorganisms exist in different ecosystems, little is known about how the individual species interact with one another, or their ecological role (their "profession" or "niche") in an ecosystem. Defining the niche is crucial, because the microbiota are a risk factor in many chronic infection scenarios (e.g., cystic fibrosis (CF) airway infections, colonization of indwelling catheters, chronic wound infections, and so on). With the ever-expanding threat of antimicrobial resistance, this information on "which each organism does" in the ecosystem is more important than ever, especially given the realization that not all members of the microbial community promote disease; some appear to prevent it. Here I introduce a computational model based on the generalized Lotka-Volterra equation and implementation of a Bayesian Inference adaptive Markov Chain Monte Carlo framework. Using time-series data obtained from the CF registry, I identified distinct ecological signatures associated with different therapeutic interventions. My machine learning approach also identified interactions that are fluid within- and between time-windows. More recently, and using a metagenomic based time-series, my approach has confirmed that different CF medications have a distinct ecological influence on the microbial interactome, and that this influence varies between hosts. My approach provides a systematic method for defining and quantifying the interactions between microbial species. My findings are leading to an entirely new way of thinking about the impact of antibiotics on multi-species microbial communities, and offer the potential to contribute towards personalizing therapeutic interventions.

A190

Genome-scale CRISPR/Cas9 knockout screening in avian cells to identify host factors essential for influenza replication

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Abstract

IAV is a critically important pathogen in terms of human health, food security and animal welfare. Despite coordinated international surveillance and control strategies, IAV regularly causes globally significant outbreaks of disease. Genome editing has the potential to generate livestock that are resistant to IAV. However, high confidence host targets that may confer resistance must be identified. Here, we performed two genome-scale CRISPR/Cas9 knockout screens in chicken lung epithelial cells (CLEC 213) with either the IAV PR8 reference strain or an avian H9N2 strain with a 3:5 re-assortant. Cells were sorted based on level of influenza infection and the guides enriched in each population were assessed using MaGECK analysis. Several guides enriched in the bottom 5% of infected cells in both screens were found to target genes involved in sialic acid biosynthesis and N-linked glycosylation. These include SLC35A1, SLC35A2, MOGS and MGAT1 in addition to the avian specific host dependency factor, ANP32A. This indicated that our CRISPR screens were successful and capable of identifying host specific dependency factors. Multiple novel hits were identified using both screens, for which the validation and characterisation is ongoing.

A191

What factors are driving bacterial colon tumor invasion? The role of cyclic adenosine monophosphate in Streptococcus gallolyticus colon cell invasion.

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Abstract

Streptococcus gallolyticus subsp. gallolyticus (Sgg) is an opportunistic Gram-positive pathogen that has been shown to colonise colorectal cancer tissues and, following colonisation, translocates through the intestinal barrier into the bloodstream causing a variety of bloodstream infections. *Sgg* were previously believed to be non-motile, however, we show for the first time that *Sgg* exhibit twitching motility and that inhibiting motility abrogates colon cancer cell invasion.

This novel motility phenotype is negatively regulated in *Sgg* in response to growth in different nucleotides, of which cAMP is the focus of this work. Increasing concentration of cAMP inhibit Sgg motility. We hypothesise that Sgg motility is conferred by a Type IV pilus, and electron microscopy shows the presence of long, thin filaments on the surface of *Sgg*. Additionally, video microscopy of *Sgg* shows the bacterial cells exhibiting twitching motility. Sgg cells move at a higher velocity and travel longer distances in the absence of cAMP.

Interestingly, colon cancer cells are known to have low levels of cAMP and cAMP is degraded by phosphodiesterases (PDEs) in colon cells. We therefore used PDE inhibitors to assess the effect of increased cellular levels of cAMP on *Sgg* ability to invade colorectal cancer cells. A variety of PDE inhibitors were tested and shown to inhibit *Sgg* invasion of cancer cells.

Based on the results from this study, we hypothesise that *Sgg* exploits the metabolic landscape of CRC tumours by inducing motility in response to a low cAMP environment. This allows Sgg to maximise nutrient acquisition, enhance colonisation and ultimately translocate through cancer cells to facilitate systemic dissemination.

A192

Impact of Allelic Diversity on Trimethoprim Resistance in Uropathogenic *Escherichia coli*

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Abstract

Urinary tract infections (UTI) are a common bacterial infection with symptoms that include urinary frequency, urgency to void, dysuria and abdominal pain. The recommended antibiotics to treat uncomplicated UTI are trimethoprim or nitrofurantoin. The incidence of trimethoprim resistance exceeds 30% in urine positive cultures. However, trimethoprim is still widely used, including prophylactic treatment of recurrent UTI.

The mode of action for trimethoprim is to competitively inhibit the di-hydrofolate reductase FolA required for co-factor synthesis necessary to produce thymine and purines. Trimethoprim resistance is driven by horizontal transfer of the gene *dfrA* that encodes a trimethoprim-insensitive di-hydrofolate reductase. There are up to 48 recognised allelic variants of *dfrA* exhibiting between 35-96% protein similarity.

Bacterial genome analysis of 283 uro-associated *Escherichia coli* isolates has identified 14 common *dfrA* alleles, Growth analysis identified an allelic bias with respect to a slow-growth phenotype, when strains were exposed to sub-MIC concentrations of Trimethoprim. Genetic replacement of the *dfrA* gene in specific isolates, was used to determine whether the identified phenotype was *dfrA* specific or related to the isolates genotypic background. The data to be presented argues that fitness correlates to *dfrA* allele carriage and could potentially be exploited to benefit the use of trimethoprim if other antibiotics are impeded by antimicrobial resistance.

This project was supported, in part, by a Harry Smith Vacation Studentship to N. Ramchandani Ramchandani

A193

Sanitary ware of Irish hospitals as a reservoir of antimicrobial resistant pathogens

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Abstract

This project monitored sanitary ware in two Irish hospitals for AMR pathogens every fortnight for three months. Shower wastewater samples and shower, sink and toilet swabs from four rooms per hospital were screened for cefotaxime and imipenem resistant *Escherichia coli* and *Klebsiella* spp., vancomycin resistant *Enterococcus* spp. (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) using culture-based methods. AMR pathogens were isolated from all sites and all rooms across all timepoints. Cefotaxime^R *E. coli* (n = 129), imipenem^R *E. coli* (n = 110), cefotaxime^R *Klebsiella* spp. (n = 105), imipenem^R *Klebsiella* spp. (n = 66), VRE (n = 22) and MRSA (n = 59) were isolated in total from shower wastewater. Shower drain swabs contained 114 cefotaxime^R *E. coli*, 49 imipenem^R *E. coli*, 98 cefotaxime^R *Klebsiella* spp., 65 imipenem^R *Klebsiella* spp., 40 VRE and 81 MRSA. Sink swabs had 114 cefotaxime^R *E. coli*, 78 imipenem^R *E. coli*, 98 cefotaxime^R *Klebsiella* spp., 73 imipenem^R *Klebsiella* spp., 20 VRE and 38 MRSA. Toilet swabs had 71 cefotaxime^R *E. coli*, 54 imipenem^R *E. coli*, 53 cefotaxime^R *Klebsiella* spp., 30 imipenem^R *Klebsiella* spp., 9 VRE and 28 MRSA. Cefotaxime^R *E. coli* was most abundant across all sample types whereas VRE was least detected. The shower aspirate samples yielded the most isolates while the toilets yielded the least. This study demonstrates hospital sanitary ware as an AMR pathogen reservoir. Hospital A had a 100% positivity rate for all sites except the toilet which was 83%. Hospital B had a 100% positivity rate for all sites.

A194

Experimental Evolution of antibiotic resistance in standard and host-mimicking media

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Abstract

Understanding the evolution of antimicrobial resistance (AMR) is integral to limiting AMR development and spread. The environment significantly impacts the adaptive landscape and influences AMR evolution through diverse selection pressures and fitness costs. Here, we use host-mimicking media and *ex vivo* biofilm models to better mimic and understand different infection sites in the host.

Antimicrobial resistant mutants of *Pseudomonas aeruginosa* were selected for in the presence of meropenem and silver sulfadiazine using an evolutionary ramp approach in synthetic wound fluid (SWF) and cation-adjusted Mueller Hinton broth (caMHB). Fitness costs and population density in the absence of antimicrobials of each the mutants were assessed comparatively in addition to replicate antimicrobial susceptibility tests. Antimicrobial susceptibility was assessed using the broth microdilution method in the respective media.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of meropenem and silver sulfadiazine (SS) were higher in SWF. Subsequently, *P. aeruginosa* was able to adapt to higher concentrations of meropenem and SS in SWF. Although, MIC, MBC and maximum selection pressure were more subtle in SS than meropenem. In both media the MIC and MBC increased as evolution continued but this was accompanied by a greater fitness cost, more so in caMHB than SWF.

We found that media can affect the antimicrobial susceptibility and evolution of *P. aeruginosa*. In the future, evolutionary trajectories will be evaluated through whole-genome sequencing and complementary experiments will be performed using *ex vivo* biofilm models to better mimic the host environment.

A195

The effect of antibiotic selection on collateral susceptibility and evolvability of uropathogenic *Escherichia coli*

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Abstract

Urinary tract infections (UTIs) are the second most common reason for prescribing antimicrobials in the UK, with *Escherichia coli* the most common cause (75% of cases) and associated with recurrence of infection (40%). Trimethoprim, nitrofurantoin and fosfomycin are recommended antibiotics for the treatment of uncomplicated UTIs, however, resistance to trimethoprim is high (33.4%). With little antimicrobial development, new strategies are required to maintain efficacy in available antibiotics. One such strategy is to understand how resistance to one antibiotic can affect the activity and probability of resistance development to a second antibiotic. Effect on antimicrobial activity can be assessed through collateral susceptibility (CS), where resistance to one antibiotic increase or decreases susceptibility to a second antibiotic. While the probability of resistance development can be assessed by the mutant selection window (MSW), an antibiotic concentration range where AMR mutations can be selected for. In this study, we aimed to determine the effect of the prior selection of trimethoprim resistance in three clinical strains of uropathogenic *E. coli* on CS and MSWs to nitrofurantoin and fosfomycin. While we found no effect in the trimethoprim-resistant mutants on CS or MSW to fosfomycin, we observed collateral resistance to nitrofurantoin. Importantly, there was a reduction in the MSW, reducing the probability of selection of higher levels of nitrofurantoin resistance. This suggests that using nitrofurantoin after trimethoprim may reduce the probability of developing nitrofurantoin resistance compared to using nitrofurantoin first, potentially providing an evidence base to use evolutionary principles when designing antimicrobial regimens to improve treatment success.

A196

Genetic diversity of polymyxin resistance mechanisms of KPC/CTX-M-producing *Klebsiella pneumoniae* clinical isolates from blood samples

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Abstract

The emergence of polymyxin-resistant bacteria is a concern in healthcare settings, as it limits treatment options for serious infections, especially for carbapenem-resistant *Klebsiella pneumoniae* (CRKP). Ten *Klebsiella spp* resistant to polymyxin B were recovered from blood samples at 10 hospitals in Sao Paulo State, Brazil. MALDI-TOF MS and *Klebsiella* MALDITypeR were used for bacterial identification. Antimicrobial susceptibility was determined by disk diffusion and minimal inhibitory concentrations (MICs) by broth microdilution according to the CLSI recommendations and/or ANVISA nº 01/2013. PCR was performed to detect bla_{ESBL} and carbapenemase genes, *tet, pmrA, pmrB, phoP, phoQ*, and *mgrB*. All isolates were identified as *K. pneumoniae*; twenty-five antimicrobials were tested and eight isolates were considered as MDR while the other two were classified as XDR. Ceftazidime-avibactam resistance was detected in one isolate. Polymyxin B MICs ranged from 12-64µg/mL while imipenem and meropenem MICs ranged from 12-≥32µg/mL, and from 0,75-≥32µg/mL; three isolates were intermediate to tigecycline. All ESBL-producing isolates according to DDST presented $bla_{CTX\text{-}M\text{-}groups\ 1\ and\ 2}$; bla_{KPC} was detected in all isolates; *tet*(A) and *tet*(B) were detected in two isolates. Mutations in PmrA, PmrB, PhoQ, and MgrB were also detected. The deletion of MgrB was presented in two isolates. No alterations were detected in PhoP. Multiple polymyxin resistance genetic defects or pathways were detected among the isolates, reinforcing the need for ongoing antimicrobial resistance surveillance. These data demonstrate that the spread of antimicrobial resistance genes encoding resistance to different antimicrobial classes, including carbapenems, polymyxin B, and tigecycline, reduces therapeutic options.

A197

Unravelling the Impact of Group B *Streptococcus* ST23 Phasevarion

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Abstract

Group B *Streptococcus* (GBS) is an important multi-host pathogen associated with neonatal sepsis and meningitis in humans, mastitis in cows, and meningoencephalitis in fish. GBS sequence type (ST) 23 is commonly found in humans, both as a colonizer of the genitourinary tract and as cause of disease, and has been isolated from warm- and cold-blooded animals. The ability of this lineage to colonize and establish a diverse array of infections indicates that it is capable of rapidly altering its expression profile to adapt to diverse niches.

Our analyses indicated that ST23 GBS are uniquely associated with a phasevarion, a dynamic type I restriction modification system predicted to regulate bacterial gene expression by modulating DNA methylation. We went on to characterise this system and were able to show that the phasevarion undergoes recombination, giving rise to distinct variants that methylate unique DNA sequences. Interestingly, these discrete populations, each expressing a unique variant, can co-exist within a single culture. We hypothesised that this heterogeneity in methylation would give rise to distinct gene expression profiles, each of which confers an advantage in a different niche.

To decipher the impact of these methylation patterns on bacterial phenotype, we generated "locked-mutants" by genetically fixing the phasevarion to express a single variant. Phenotypic characterization of these mutants has identified variants associated with enhanced fitness in different niches, providing a clear rationale for the molecular mechanism underlying the ability of ST23 GBS to cause a diverse array of disease manifestations within human and animal hosts.

A198

Antibacterial efficacy of leaf extracts of Acacia nilotica and Jatropha curcas against multidrug resistant Pseudomonas aeruginosa and Staphylococcus aureus clinical and environmental isolates.

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Abstract

The increase in the emergence of multidrug resistance strains of pathogenic bacteria worldwide has necessitated the search for novel antimicrobial agents, especially from natural systems like plants. This study aimed at evaluating the antibacterial potential of *Acacia nilotica* and *Jatropha curcas* against multidrug resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *A. nilotica* and *J. curcas* leaves were extracted using chloroform and phytochemical contents of the plant extracts were determined. One hundred and fifty clinical and environmental samples each, were collected for the isolation of *P. aeruginosa* and *S aureus*. The antibiogram profile of the isolated strains was ascertained using the standard disc diffusion bioassay. Antibacterial potential of the plants extracts on the most resistant strains of the two bacterial isolates was assessed in-vitro. The identities of the multi-drug resistance isolates were confirmed by PCR and sequencing of their 16SrDNA. Antibiotic resistance genes (AcraA, blaOxa, blaVIM, blaTEM, CTXM and blaSHV) were detected in *P aeruginosa* isolates using multiplex PCR. Tannins, saponin, alkaloids, flavonoids, phenols, terpenoids, quinones, phytosterols were present in the plants extracts. *In-vitro* bioassay of the plant extracts against the multidrug resistant bacterial isolates revealed that *J. curcas* extracts (1000µg/ml) had the highest zones of inhibition at 20mm compared to two standard antibiotics used, Levofloxacin and gentamycin at 17mm and 14mm respectively. Combination of the plants extracts exhibited even more activity against the resistant strains of both bacteria with maximum inhibition zone of 31mm. The tested leaves extracts can be potential source of antibacterial drug against multidrug resistant bacteria.

A199

TLR3-induced Inflammation in Avian Epithelial Cells Enhances the Invasion Profile of *C. jejuni*

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Abstract

Extra-intestinal spread of *C. jejuni* in broiler chickens and dissemination to edible tissues represents a significant public health threat. There is good *in vivo* evidence in broiler chickens that *C. jejuni*-induced inflammation may be responsible for compromising epithelial cell barrier integrity and may be a pre-requisite for extra-intestinal spread. Understanding the specific inflammatory stimuli that link *C. jejuni,* the gut epithelium and inflammation is integral in designing intervention methods for *Campylobacter* control. Using cell culture, 8E11 avian intestinal epithelial cells were treated with various pattern recognition receptor (PRR) agonists or cytokines to induce defined local immune responses prior to treatment with *C. jejuni* for 4 hours. Epithelial cells were lysed for bacterial enumeration (gentamicin protection assay [GPA]) or quantitative RT-qPCR to determine expression of avian cytokines. Pre-treatment with the intracellular agonists Poly I:C (TLR3) or MDP (NOD-2) significantly enhanced invasion of a “low invasion” *C. jejuni* strain (LE17) compared with “high invasion” LE55 and M1 control. Additionally, Poly I:C treatment led to significantly increased levels of CXCLi2 (IL-8) only in avian cells treated with *C. jejuni,* an effect that was not replicated in independent treatments. This interaction was later found to be partially mediated through the NLRP3 inflammasome. Together, these results suggest that the type of gut inflammation experienced by broiler birds is integral to the ability of *C. jejuni* to invade gut cells and recruit blood derived phagocytes. Anti-inflammatory strategies must therefore focus on the type of inflammation generated in order to gain specific control of *Campylobacter.*

A200

Characterisation of a KPC-2 mutant inducing antimicrobial resistance gene silencing and conferring resistance to avibactam in clinical Escherichia coli.

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Abstract

Antimicrobial resistance (AMR) prediction from whole genome sequencing (WGS) is growing in interest to be used as a rapid diagnostic to select the correct antibiotic to successfully treat an infection. Phenotype-genotype discordance, wherein the predicted presence or absence of an AMR gene does not match the realised phenotypic resistance, compromises this strategy. It is therefore important to identify potential phenotypic-genotypic discordance to improve and maximise the utility of AMR prediction.

Here, we used a random mutagenesis/error prone PCR approach to induce single nucleotide polymorphisms (SNPs) in the carbapenemase bla_{KPC-2} to confer resistance to ceftazidime-avibactam (CZA). SNPs were identified using plasmid sequencing of the CZA resistant library, and a novel, low-frequency SNP, Thr242Pro, was discovered. bla_{KPC-2} with this SNP was transformed into clinical *Escherichia coli* isolates, which were then phenotypically characterised through antibiotic disc diffusion, fitness, and nitrocefin assays.

We found that the mutation not only conferred resistance to CZA, while remaining resistant to cephalosporins, but also 'silenced' resistance to imipenem and meropenem, rendering it clinically susceptible. Furthermore, isolates with the mutated KPC-2 could no longer hydrolyse nitrocefin, despite maintaining cephalosporinase capabilities.

In this study with have identified a novel SNP in bla_{KPC-2} which alters the resistance profile. Additionally, we have highlighted the importance of identifying and characterising instances of phenotype-genotype discordance which may complicate the effectiveness of resistance prediction using WGS.

A201

Tracking the spread of SARS-CoV-2 variants using serology

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Abstract

Introduction: Since the beginning of the COVID-19 pandemic, numerous variants of concern (VOC) have emerged including Alpha (B.1.1.7) and Omicron (B.1.1.529). Mutations in the spike protein make these VOCs more transmissible and affect neutralisation elicited by vaccination, and therefore tracking variants is essential to monitor SARS-CoV-2 outbreaks within the population. Here, we detail a multiplex SARS-CoV-2 serology assay for measuring SARS-CoV-2 variant-specific antibody responses and utilise these to track VOC spread within healthcare workers.

Methods: Serum samples were taken from ~1,500 healthcare workers enrolled into the SIREN study, all with PCR-confirmed SARS-CoV-2-infections between October 2020 to February 2021. Multiplex SARS-CoV-2 serology immunoassays were used to measure variant-specific spike and RBD antibody binding responses, with ROC analysis to determine assay sensitivity, specificity, and optimum assay cut-offs.

Results: The spike antigen demonstrates a high sensitivity at 97.14% in discriminating between Wuhan and Alpha-infected individuals, with a 90.80% specificity. However, RBD showed poor sensitivity (77.14%) and specificity (77.01%). Utilising the spike antigen, serum samples from SIREN participants with a PCR-confirmed infection during the Alpha wave were analysed, with VOC-specific seroreactivity mirroring temporal changes in national sequencing data.

Discussion: This multiplex SARS-CoV-2 serology assay demonstrates capability at reliably identifying VOC-specific antibody responses in non-vaccinated individuals with singular SARS-CoV-2 infection. The impact of Alpha infection and antigenic imprinting on future antibody responses after vaccination is being explored further. This shows the reliability of multiplex-serology for serotyping, both for inferring infection type based on antibody binding without sequencing and determining VOC spread such as through serosurveillance.

A202

The role of non-typeable *Haemophilus influenzae* in cystic fibrosis lung infection

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Abstract

Non-typeable *Haemophilus influenzae* (NTHi) colonises the lungs of people with cystic fibrosis (CF) in infancy. An understanding of early lung colonisers may become increasingly important as modulator therapy progresses, which could stop patients progressing to “classic” chronic infection with later stage pathogens, like *Pseudomonas aeruginosa*. There is currently little research on NTHi lung infection in CF, compared with later stage pathogens.

I am using an *ex-vivo* pig lung model as a high validity model for respiratory infections, combined with synthetic CF sputum media (SCFM) to mimic the nutritional composition of CF sputum. This is the first time this model has been used for NTHi. I aim to answer key questions about how NTHi infection affects CF lung disease progression including:

- What is the biofilm structure of NTHi on host lung tissue?
- Does the growth of NTHi as a biofilm in the lungs affects susceptibility to standard antibiotic treatment?
- Does NTHi alter the lung environment to assist other CF pathogens to colonise the lungs?

I have optimised the formulation of SCFM for use with NTHi, determining that supplementation with additional molecules found in CF lung mucus is required for biofilm growth *in vitro*. This was assessed through growth curves and static biofilm assays. Using this supplemented SCFM, NTHi biofilm forming ability, biofilm structure and antibiotic susceptibility can be assessed in the lung model. I will use this optimised model to better understand the physiology and ecology of early-stage pathogen infection in CF, and elucidate the role of NTHi in CF disease progression.

A203

Exploring the microbiome and resistome of domestic laundry machines

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Abstract

On site domestic laundry machines (DLM) in healthcare settings have been previously identified as a source of infection. However, little is known about the potential role of DLM in transferring potentially pathogenic bacteria and antibiotic resistance genes. Increased knowledge of the microbiome and the resistome of DLM is of particular importance due to healthcare workers regularly using their DLM to clean and disinfect their uniforms within the home environment. The aim of this study is to explore the microbiome and resistome of DLM in order to begin to understand their potential role in the transmission of pathogens and antibiotic resistance.

Shotgun metagenomic analyses was performed on eight DLM (detergent pipe and rubber rim). In addition, the presence of potentially pathogenic bacteria and specific antibiotic resistance genes was detected by qPCR on an extended set of samples.

Preliminary metagenomic analyses of the bacteria composition showed that three main bacteria classes were present in the samples: Actinomycetes, Gamma-proteobacteria and Alpha-proteobacteria. They contribute to more than 80% of the reads for more than 75% of the samples. Potentially pathogenic genera were present such as *Mycobacterium* and *Pseudomonas*. The presence of antibiotic resistance genes was detected in all the samples including efflux pump (adeF), target modification (Van) and antibiotic inactivation genes (ANT 3''IIC).

The metagenomic analysis confirmed the presence of antibiotic resistance gene containing bacteria in DLMs. The potential presence of those bacteria on domestically laundered healthcare textiles could be a transmission route in the clinical and domestic environment and requires further investigation.

A204

Dynamics of macrophage phagocytosis: *in vitro* and *ex vivo* models

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Abstract

Streptococcus pneumoniae is a major pathogen that causes severe human infections and sepsis. A crucial role is played by the tissue resident macrophages which provide innate cellular immunity. Studies in mice showed that invasive disease occurs after rare macrophage failure leading to a within-macrophage replication. We focused on the mechanisms by which pneumococci survive professional phagocytes.

To describe intracellular survival, we performed invasion/adhesion and killing assays and time-lapse confocal-microscopy of unencapsulated FITC-tagged pneumococci and RAW 264.7 macrophages. Moreover, to characterize the function of human tissue macrophages, *ex vivo* human spleen perfusions were performed.

In vitro pneumococcal phagocytosis showed that almost all pneumococci were taken-up by macrophages with a killing rate of 85/90%. Moreover, time-lapse confocal microscopy image analysis indicated that over 80% of macrophages took-up multiple pneumococci with average of 13 bacteria per macrophage and cell-lysis of 20%.

The *ex vivo* human spleen perfusions showed that the organ removes pneumococci from the perfusion liquid, with 90% of bacteria disappearing in the first hour. Immunohistochemistry indicates that pneumococcal killing may-be complemented by macrophages apoptosis. Analysis showed that the organ is composed by 19% of CD163+ macrophages, that 1/400 macrophage did phagocytize a pneumococcus and that 0.5% of CD163+ macrophages is apoptotic; meaning that each macrophage that phagocytized a bacterium dies.

The data indicate that bacteria appear to survive in some phagocytes and bacterial killing appears to induce cell-death. We are re-focusing our questions in asking what happens in the very rare cells where efficient killing is blocked leaving alive bacteria within phagocytes.

A205

Improving survival in premature infants: development of feeding tubes with a biofilm resistant coating

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Abstract

Biofilm formation on the surfaces of medical devices is a leading cause of hospital-acquired infections among premature neonates. Up to 56% percent of neonatal deaths can be related to these infections and surviving babies are often affected by life-long disabilities. Feeding tubes are among the most widely used medical devices in neonatal intensive care. Up 89% percent of feeding tubes removed from infants are colonised by bacteria, in some cases as early as one day after insertion of the device. Feeds provided through contaminated tubes can lead to gastrointestinal or systemic infections, which can ultimately evolve into fatal outcomes. Here we describe the development of neonatal feeding tubes coated with a biocompatible, non-biocidal anti-biofilm polymer. Commercially available polyurethane nasogastric paediatric feeding tube segments were dip coated into polymer solutions. The coating characteristics were assessed using optical profilometry. Biofilm formation of clinically relevant bacterial species on the samples was investigated with scanning confocal microscopy. The performance of the coated tubes was also assessed under milk conditioning. The polymers were successfully demonstrated to form uniform coatings of approximately 20 µm thickness on the substrate, exhibiting good mechanical durability. Tubes coated with poly(isobornyl acrylate-co-diethylene glycol methacrylate) 60:40 demonstrated the best overall bacterial biofilm resistance in vitro, with an average reduction of 89% compared with uncoated tubes for *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Our findings demonstrate the potential for the development of a new coated device that could prevent biofilm-related infections among high-risk premature infants.

A206

Ex vivo human spleen perfusion model to study pneumococcus-macrophage interactions

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Abstract

Tissue resident macrophages are a heterogeneous population that provides innate cellular immunity removing pathogens from the bloodstream. *Streptococcus pneumoniae* is one of the major causes of community acquired pneumonia, which can lead to sepsis. A major unanswered question is how pneumococci sustain bacteraemia that develops into sepsis. *S. pneumoniae* is exposed to phagocyte-mediated clearance mechanisms in the spleen but the exact role of spleen macrophages is still unknown.

To better investigate the pneumococcus-tissue macrophages interaction, we infected *ex-vivo* perfused human spleens with a mix of different pneumococcal strains. Biopsies taken at different time-points were analyzed through immunohistochemistry, aiming to correlate the bacterial clearance with macrophage activation. The activated-lysosome marker used during the microscopy was Lamp1.

The analysis of the immunohistochemistry images showed that CD169+ macrophages harbour 10x more bacteria than CD163+ and that the number of bacteria within CD169+ cells decreased over time. Moreover, almost all the Lamp1 cellular marker was detected within macrophages. Lamp1 showed an increase over the first 30 minutes of infection and then decreased over time. A similar trend was followed by the total bacteria count. Furthermore, we stained the high-virulent T4 and the low-virulent 19F serotypes separately, in order to evaluate whether they exhibited different behaviors. No significant differences were observed.

In conclusion, the *ex vivo* perfusion of human spleens is allowing us to functionally characterize human tissue macrophages. The data obtained so far indicate that also in humans the replication in permissive CD169+ macrophages could be critical for the start of invasive infections.

A207

A Slice of Home: Using Artificial Sputum Medias to Cultivate the Cystic Fibrosis Lung Microbiome

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Abstract

Advancements in cystic fibrosis (CF) treatment have drastically extended the lifespans of people with CF; however, periodic pulmonary exacerbations in part caused by chronic bacterial infection are still the leading cause of morbidity and mortality. Additionally, treatment requires prolonged antibiotic use which potentiates antimicrobial resistance, a global healthcare concern. Recent studies have revealed the complexity of the CF lung microbiome, where microbial composition/interaction affect disease progression. Previous work indicates that culturing sputum on commercial agars recovers ~99.3% of the relative abundance of this community. Artificial sputum medias (ASM) mimic the nutritional composition of the CF lung environment; to-date, these have predominantly been used to culture the principal pathogen *Pseudomonas aeruginosa*. This prompts the investigation into whether ASM can culture the lung microbiome more effectively than commercial agars.

This study focusses on two common ASMs (ASM, ASMDM) and mucin varieties (Type II, III) to determine their ability to culture the lung microbiome compared to commercially available solid agars. Both frozen and fresh sputum samples were cultured on each commercial agar and ASM variation. ASM-Type II consistently cultured less phenotypic diversity than the other agars, whereas ASMDM-Type III had similar phenotypes and CFUs to many commercial medias. Ongoing 16S rRNA gene sequencing analysis is expected to reflect ASM's ability to cultivate a subset of microbes compared to commercial agars and that these results are influenced by the type of mucin used. A better understanding of ASM's ability to culture the CF lung microbiome will inform further investigations of this clinically-important community.

A208

Clinical Presentation and Outcomes of Critically Ill Patients with Difficult to treat (DTR) Pseudomonas septicemia treated with Cefiderocol- A Case Series

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Abstract

Introduction: Cefiderocol, a novel siderophore cephalosporin, has recently become a valuable addition to our arsenal of antibiotics for addressing severe infections caused by drug-resistant gram-negative organisms. However, it is currently unavailable in India. In this context, we present our experiences with Cefiderocol in treating challenging Pseudomonas aeruginosa infections observed in four patients.

Material and Methods:

Included in this series were patients with infections attributed to drug-resistant (DTR) Pseudomonas aeruginosa, unresponsive to conventional antibiotic therapy. The treatment cohort consisted of individuals administered Cefiderocol between January and July 2023. DTR was characterized by nonsusceptibility to all antipseudomonal antibiotics.".

Results:

Our case series comprised four patients, with a demographic distribution of 2 (50%) males, 3 (50%) individuals aged over 75 years, and all 4 (100%) being immunocompromised. In each case, Pseudomonas aeruginosa infection was identified, and all proved to be extensively drug-resistant. The isolates exhibited resistance to Ceftazidime-Avibactam, Carbapenems, Amikacin, and Fosfomycin, while maintaining in vitro susceptibility to colistin. Due to suboptimal clinical responses to colistin or a ceftazidime-avibactam-aztreonam regimen, Cefiderocol was initiated. The treatment duration ranged from 14 to 28 days, determined by the severity of the infection and the underlying etiology. Encouragingly, all four patients exhibited recovery from their illnesses.

Conclusion:

Cefiderocol, with established efficacy against drug-resistant gram-negative organisms, presents itself as a promising alternative for treatment in situations where our antibiotic choices are limited.

A209

Developing a 3D platform to investigate the role of *Fusobacteria* in ovarian cancer

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Abstract

The *Fusobacterium* genus, comprising Gram-negative, obligate anaerobic bacteria found in the oral cavity, gastrointestinal, and female genital tracts, is associated with pathologies such as chronic periodontitis and inflammatory bowel disease. *Fusobacteria*, particularly *Fusobacterium nucleatum*, have a predominant role in carcinogenesis, facilitated by surface adhesins like FadA, Fap2, and CbpF. These adhesins bind to receptors on malignant cells, principally E-cadherin, Gal-GalNAc and CECAMs, respectively, promoting malignant behaviours.

Fusobacterial colonisation has been observed in various cancers, initially in colorectal cancer and subsequently in head and neck, pancreatic, ovarian, cervical, and breast cancers. Elevated intratumoral Fusobacteria levels correlate with poorer progression-free and overall survival rates in ovarian cancers. Moreover, sensitivity to neoadjuvant chemotherapy aligns with intratumoral Fusobacterial colonisation, suggesting a role in mediating therapeutic responses.

Understanding the intricate relationship between the microbiome and malignant cell behaviour is crucial. The development of 3D tumour models enables better recapitulation of the tumour microenvironment and cell-cell interactions. Therefore, a 3D tumour spheroid model using ultra-low attachment plates was developed to elucidate the *Fusobacterial* influence on carcinogenesis in a more physiologically relevant system than traditional monolayer culture. This 3D model revealed significant expression level changes in cancer cell ligands responsible for engaging with *fusobacterial* adhesins.

A212

Nucleoside analogues as anti-bacterial agents targeting pathogenic *Escherichia coli*

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Abstract

Intestinal pathogens enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E.coli* (EHEC) cause diarrhoeal disease in children. An unpublished screen of 1120 drugs assaying human cell infection by EPEC revealed FDA-approved nucleoside analogues that abrogated infection. Initial experiments indicate that the nucleoside analogues have novel antibiotic activity towards EPEC/EHEC. Though we do not understand the antibiotic mechanism, it is possible that the nucleoside analogues are incorporated into elongating DNA chains causing damage and growth arrest. However, EPEC/EHEC overcome the nucleoside analogues through an unknown antimicrobial resistance mechanism. The nucleoside analogues in question are oral chemotherapies for child leukaemia. We found that EPEC antimicrobial resistance inactivates the nucleoside analogues, which no longer activates DNA damage and apoptotic responses in human cancer cells.Thus, the project is of significance to antimicrobial research and cancer treatment regimes, particularly as bacteria in the microbiome may also inactivate the nucleoside analogues.

A213

Developing host-mimicking growth media to replicate Pseudomonas aeruginosa biofilm infection environments: a focus on cystic fibrosis and ventilator associated pneumonia.

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Abstract

Pseudomonas aeruginosa (PA) biofilm infections are strongly associated with antibiotic resistance and are difficult to treat, contributing to high morbidity and mortality in people with cystic fibrosis (CF) and ventilator associated pneumonia (VAP). Because standard antibiotic susceptibility tests do not replicate the infection environments well, CF and VAP patients often experience chronic PA lung infections, which reduce quality of life and put pressure on healthcare services.

I have compared the growth and biofilm production of PA laboratory strains PA14 and PAO1 and novel CF and VAP clinical strains *in vitro* in a selection of CF and VAP host-mimicking growth media. I used three versions of synthetic CF sputum media (SCFM) and a novel synthetic airway surface liquid (SASL) to mimic VAP conditions. Growth profiles assessed using 24-hour growth curves and static 48-hour biofilm assays. I observed growth and biofilm production for all strains tested in all types of host-mimicking media used. I also found significant differences in the growth and biofilm production of PA between different types of medium, including an increase in the growth in our modified SCFM.

Next, I will investigate the growth and biofilm production of laboratory and clinical PA strains in CF and VAP host-mimicking biofilm models: our *ex vivo* pig lung model of the CF lung and *in vitro* endotracheal tube model of VAP. This will contribute to SCFM and SASL development, with which we can advance our understanding of PA biofilm infections and improve diagnosis and selection of appropriate antimicrobial therapy.

A214

Prevalence of trichomoniasis and gonorrhoea amongst pregnant women and the associated risk factors

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Abstract

Untreated trichomoniasis and gonorrhoea in pregnancy are associated with a high risk of miscarriages, premature births and babies with low birth weight. Also, vaginal delivery increases the risk of gonococcal eye infections in the new-born that may lead to blindness. The study was carried out to determine the prevalence and possible risk factors for *Trichomonas vaginalis* and *Neisseria gonorrhoea* in pregnant women in Aba metropolis of Abia State, Nigeria.

Urine and vaginal swab samples were obtained from 132 pregnant women aged 15 to 49 years. *T. vaginalis* was detected via microscopy while *N. gonorrhoea* was identified by culture methods and confirmed using biochemical tests. The susceptibility of gonococcal isolates to anti-gonococcal antibiotics was tested using the Kirby-Bauer Disc Diffusion Assay.

The prevalence of *T. vaginalis* and *N. gonorrhoea* is 9.09% and 5.30% respectively. All gonococcal isolates were resistant to cefuroxime, cefotaxime, cefixime and ceftriaxone. 43% and 29% of the gonococcal isolates showed intermediate and resistant phenotypes respectively to ofloxacin. Treatment with acridine dye did not improve susceptibility to these antibiotics showing that resistance observed may not be plasmid-mediated. Variables independently associated with *T. vaginalis* and *N. gonorrhoea* infections in this study are the history of miscarriages (Odds Ratio (OR) = 0.32; p = <0.001), stillbirth (OR = 0.67; p = 0.025) and previous gonorrhoea or urinary tract infection (OR = 1.06; p = 0.031).

This study identifies the importance of monitoring infectious diseases in pregnancy. Early diagnosis and intervention can reduce the burden of foetal death and congenital complications.

A215

Protectors gone rogue: Pathological neutrophil activity underpins mouse susceptibility to *Citrobacter rodentium* infection

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Abstract

Why do some people get severely ill when infected, whilst others do not? In order to address this question, and study host factors underlying disease severity, we employed *Citrobacter rodentium* (CR), a natural mouse-adapted pathogen that is used to model infectious and ulcerative colitis (UC). While C57BL/6 (C57) mice display mild symptoms and clear CR, C3H/HeN (C3H) mice succumb to infection, which correlates with increased luminal neutrophil elastase activity. Neutrophil recruitment was significantly higher in the colon of infected C3H mice, coinciding with tissue damage, signs of dehydration and heightened serum G-CSF levels. Examination of bone marrow (BM) neutrophils revealed that C57 BM neutrophils undergo extensive reprogramming and display increased levels of activation markers upon CR infection, while no major changes were seen in C3H BM neutrophils, which just increase in number. Accordingly, the number of blood and colon neutrophils were ~2 fold higher in C3H mice; however, C3H neutrophils failed to reach the infection site, instead accumulating in the submucosa where they release their pro-inflammatory contents, contributing to tissue pathology. Infection with a CR mutant triggering a localised hyper-inflammatory state led to reduced morbidity and C3H survival, coinciding with increased migration of neutrophils to the infection site. Importantly, UC patient blood neutrophils also displayed decreased levels of CD16, a neutrophil activation marker. This study uncovers novel neutrophil pathologies during colitis in mice and humans, which we hope will open the door to more targeted therapies that can reduce neutrophil-related tissue damage without impacting on their antimicrobial capabilities.

A216

Deception point - Tuberculosis or Nocardiosis ? : An Indian Case Series

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Abstract

INTRODUCTION

Nocardiosis is a disease afflicting the immunocompromised, notorious for mimicking infections like tuberculosis. Its diagnosis is hence challenging in countries with a high tuberculosis burden, warranting astute clinical and microbiological practices.

METHODS

We studied four patients who sought care at our tertiary facility between January 2020 and June 2023, displaying symptoms akin to tuberculosis but were subsequently diagnosed with Nocardiosis.

RESULTS

The study included 3 males (75%) and 1 female (25%). 3 had identifiable immunocompromising attributes, including corticosteroid use(100%), recent chemotherapy(33.3%) and diabetes mellitus(66.6%). All were HIV seronegative with no transplant history. 3(75%) had pulmonary disease and 1 (25%) had CNS involvement. Nodulo-cavitatory lung lesions and multiple ring-enhancing brain lesions were significant radiological findings. Molecular TB tests were negative in all patients. Subsequent cultures using the Matrix-Assisted-Laser-Desorption-Ionization-Time-of-Flight Mass-Spectrometry (MALDI-TOF MS) isolated N. beijingensis(66.6%) and N. cyriacigeorgica(33.3%) in pulmonary disease, and N. farcinica in the patient with CNS lesions. Trimethoprim-Sulphamethoxazole was universally susceptible and other drugs employed were Meropenem and Amikacin. 1 patient developed cytopenias while on Trimethoprim-Sulphamethoxazole, hence Linezolid was used as an alternative. Treatment duration varied between 6-12 months and all patients successfully completed treatment, achieving clinical and radiological resolution on follow up.

CONCLUSION

Nocardiosis is almost indistinguishable from tuberculosis, entailing underdiagnosis of infection and co-infection in high tuberculosis-incident countries like India. Ongoing cell-mediated immunity suppression such as corticosteroid use, should instigate a hunt for nocardiosis. MALDI-TOF MS is a powerful tool in enabling early diagnosis and therapy initiation in Nocardiosis.

A217

Longitudinal analysis within one hospital in sub-Saharan Africa over 20 years reveals repeated replacements of dominant clones of *Klebsiella pneumoniae* and stresses the importance to include temporal patterns for vaccine design considerations

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Abstract

Infections caused by multidrug-resistant gram-negative bacteria present a severe threat to global public health. The WHO defines drug-resistant *Klebsiella pneumoniae* as a priority pathogen for which alternative treatments are needed given the limited treatment options and the rapid acquisition of novel resistance mechanisms. Longitudinal descriptions of genomic epidemiology of *K. pneumoniae* can inform management strategies but data from sub-Saharan Africa are lacking, despite the severe public health burden caused by *K. pneumoniae* infections, in particular causing neonatal sepsis.

We present a longitudinal analysis of all invasive *K. pneumoniae* isolates from Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, southern Africa, which has undertaken sentinel surveillance of bacteraemia and meningitis in partnership with the Malawi-Liverpool Wellcome Programme (MLW) since 1998. We combine clinical data with genome sequence analyses spanning over two decades, providing an unprecedented longitudinal insight in this understudied subcontinent. We show that after a dramatic increase in infections from 2016 *K. pneumoniae* becomes hyperendemic, driven by an increase in neonatal infections. Genomic data show repeated waves of clonal expansion of different, often ward-restricted, lineages, suggestive of hospital associated transmission. We describe temporal trends in resistance and surface antigens, of relevance for vaccine development.

Our data highlight a clear need for new interventions to prevent rather than treat *K. pneumoniae* infections in our setting. Whilst one option may be a vaccine, most cases could be avoided by an increased focus on and investment in infection prevention and control measures, which would reduce all healthcare associated infections and not just one.

A220

Proteomic profiling of primary ciliary dyskinesia airway epithelia infected with non-typeable *Haemophilus influenzae* biofilm

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Abstract

Introduction

Primary ciliary dyskinesia (PCD) is an inherited ciliopathy, in which poor ciliary function and low nitric oxide levels are thought to increase airway epithelium susceptibility to non-typeable *Haemophilus influenzae* (NTHi) biofilm infection. Beyond these factors, other host intrinsic factors potentially contribute towards increased NTHi infection but are understudied.

Aim

Differential protein expression in PCD airway epithelia was investigated to identify dysregulated biological processes that contribute towards increased NTHi biofilm infection.

Methods

Primary airway epithelia from donors with (n=6) and without PCD (healthy; n=3) were cultured at air-liquid-interface (ALI) for 4-6 weeks. ALI-cultures were then infected with a PCD NTHi isolate at multiplicity of infection 50 and co-cultured to form biofilms over 3 days. Label-free proteomics was used to quantify relative protein abundance and differentially expressed proteins analysed using Gene Ontology Protein ANalysis Through Evolutionary Relationships (PANTHER) to determine biological processes.

Results

We identified 201 significant differentially expressed proteins in response to NTHi biofilm infection. Unique to PCD were 51 up- and 35 down-regulated proteins, compared with healthy epithelia. Notably, 24 proteins implicated in metabolism were enriched; as were S100, plakin and actin filament proteins (cytoskeletal organisation), and MUC5AC (hyperconcentrated in muco-obstructive diseases). Expression of ICAM-1, a major human receptor involved in NTHi clearance, was also diminished.

Conclusion

Our combination of ALI-co-culture with label-free proteomics captured aberrant PCD cellular functionality in response to NTHi biofilm infection. Metabolic hyperresponsiveness, structural instability and hindered NTHi clearance may explain the increased susceptibility of the PCD airway to NTHi biofilm infection.

A222

Phenotypic and Genotypic diversity in Moraxella catarrhalis: Using Population Biology to Understand Antimicrobial Resistance and Pathogenesis

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Abstract

Moraxella catarrhalis (Mx) is a gram negative, opportunistic pathogen which colonises the human respiratory tract. Mx has the ability to cause a variety of infections including middle ear infections in children, exacerbations of Chronic Obstructive pulmonary disease, pneumonia and sepsis[1,2,3]. With a growing number of infections and rapid spread of Antibiotic resistance in Mx populations, it is vital to understand more about this underappreciated pathogen[4,5]. This project will identify the extent of both the phenotypic and genotypic variation in a large population of Mx clinical isolates. A range of phenotypes key to Mx pathogenesis will be explored including antibiotic resistance and biofilm formation alongside Multi Locus Sequence Typing (MLST) analysis.

A particular focus so far, however, have been the outer membrane adhesins and their role in adherence to human epithelial cells. A group of proteins known as UspAs mediate Mx's adhesion to a range of cell types via CEACAMs (Carcinoembryonic antigen-related cellular adhesion molecules) found on the surface of human epithelial cells[6]. Here, a variant UspA protein is shown to provide the bacterium with significantly higher levels of binding to human CEACAM1 through Enzyme linked immunosorbent assays. MLST data has confirmed this group of high binders are also genetically distinct from other strains. This finding allows us to infer more about the evolution of the species, including its two distinct Seroresistant and Serosensitive lineages. Variation in another important outer membrane protein, Hag, is also explored with results pointing towards a second, as yet unidentified CEACAM binding ligand in the population.

A224

Evaluating the Efficacy of the Type III Secretion System Inhibitor, Aurodox, against a Murine Model of Shiga-Toxin Producing E. coli (STEC).

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Abstract

Shiga Toxin-Producing *E. coli* (STEC) is an acute pathogen of the small intestine which is responsible for foodborne outbreaks of bloody diarrhoea. STEC infections are often associated with high morbidity and mortality rates due to the production of Shiga toxins (Stx) which can initiate Haemolytic Uremic Syndrome (HUS), a major cause of acute renal failure in children. Unlike most bacterial infections, STEC cannot be treated with traditional antibiotics as DNA damage induced by these treatments can activate the bacterial SOS response, which in turn leads to the induction of phage-encoded Stx. In our previous studies, we have investigated aurodox, a natural product of *Streptomyces goldiniensis*- as a potential anti-virulence therapy for the treatment of STEC. To understand its mechanism of action and assess the suitability of this molecule for repurposing, a multidisciplinary approach to understanding aurodox was used. Whole transcriptome analysis, cell infection and GFP-reporter assays were used to demonstrate that aurodox transcriptionally downregulates the expression of the Type III Secretion System (T3SS)- an essential colonisation factor in STEC. Recently, we have established a *Citrobacter rodentium* + Stx murine model to study the efficacy of aurodox treatment against Stx-positive infections. Here, we have shown that aurodox protects mice against HUS-associated phenotypes including weight loss and damage to the tubular networks of the kidney, resulting in a 50% increase in survival. In addition, we have studied the effects of aurodox treatment on the gut microbiome of mice, demonstrating that aurodox induces a bloom in the probiotic strain *Bifidobacterium animalis.*

A225

Transferability and overexpression of the intrinsic regulator RamA.

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Abstract

Inter-species chromosomal AraC-type regulators, exemplified by RamA, play a pivotal role in modulating antibiotic susceptibility in response to external stressors. Recent research has unveiled the localization of the ramA gene on the accessory genome of the IncHI2 pR18.0877_278k plasmid in *Salmonella Enterica subs. Enterica serovars Goldcoast*.

Our study was initiated by constructing a comprehensive plasmid library containing the RamA element, employing the search term 'RamA AND plasmid' subsequently, an nBLAST analysis utilizing plasmid-borne *ramA* expanded this library. To elucidate the dynamics of *ramA* transposition, we scrutinized the immediate flanking regions of the *ramA* gene in plasmids, constructing a transposition cassette, and employed plasmidfinder to discern incompatibility groups. These data were integrated into a transposition and recombination model, elucidating the stabilization of RamA in plasmids. Our biological validation involved plasmid conjugation frequency rate experiments and *ramA* cloning, demonstrating its transferability and functionality across three distinct species (*K. pneumoniae, S. Enterica, E. coli*).

Remarkably, our search identified over 100 *ramA*-carrying plasmids. Furthermore, our results underscored the prominent roles of IS1380 and IS6 elements in mediating the translocation of chromosomal *ramA* to plasmids. Notably, the absence of either or both elements correlated with specific plasmid types, IncHI and IncF, respectively. We established transferability rates and specific inducing conditions for plasmid transfer, enhancing our understanding of the role of plasmid borne-RamA in antibiotic resistance and virulence modulation.

A226

Targeted ROS Amplification in UPEC Infection

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Abstract

Uropathogenic *Escherichia coli* (UPEC) cause 80% of urinary tract infections (UTIs) globally. In the UK, there is a £400 million burden on the NHS to control infections each year. The urinary tract is the origin of infection for 25% of sepsis cases, and in Wales most cases are caused by *E. coli*. Dampened immunity within the urogenital tract often does not eradicate UTIs resulting in recurrent infection in 25% of cases. Neutrophils are major effector cells in the immune response to UPEC and their subsequent oxidative burst is critical in killing urogenital pathogens. However, UPEC possess numerous virulence factors (e.g. OxyR, RpoS) which provide UPEC with enhanced resistance to oxidative stress, contributing to the development of recurrent infections. This research aims to assess how targeted ROS amplification via FPR1 can impact the pathogenicity of UPEC in an *ex vivo* bladder model, using unique compounds provided by ProNoxis. We have isolated blood culture positive UPEC from the Hywel dda health Board, that display a range of tolerances towards oxidative stress. The impact of ROS amplification will be modelled using neutrophils isolated from healthy donors and bladder epithelial cells (SV-HUC-1), and subsequently assessed with light microscopy, microbial killing assays, and invasion assays. The mechanism of killing will be assessed using ELISA to determine cytokine concentrations and LC-MS to determine oxysterol profiles. This research will explore novel targets for UPEC which could serve to complement conventional antibiotics and reduce the development of antimicrobial resistance.

A227

Bloodstream infection-associated dysregulation of haemostasis can be modulated by iron-regulated Staphylococcus aureus cell wall proteins

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Abstract

Staphylococcus aureus is a leading cause of bloodstream infections and a significant portion of the morbidity associated with infection can be attributed to the ability of *S. aureus* to dysregulate haemostatic functions through interaction with platelets via Microbial Surface Components Recognising Adhesive Matrix Molecules (MSCRAMMs). These cell wall-anchored proteins have been widely studied in nutrient-rich media, but information about their function in nutrient-limited media, such as blood plasma, is limited. As such, we have studied the effect of iron limitation on the ability of *S. aureus* to modulate platelet function *in vitro*, with a particular focus on the haem-uptake machinery, Isd. *S. aureus* mutants lacking cell wall-anchored Isd proteins, IsdB and IsdA, were precultured in low-iron conditions. Cells were then introduced to platelet-rich plasma and subsequent platelet aggregation was measured using light-transmission aggregometry. Our data suggests an iron-dependent effect on the lag time between bacterial inoculation and initiation of platelet aggregation, as well as the time for platelets to reach maximum aggregation. Finally, our data suggests that the primary protein with which *S. aureus* initiates platelet aggregation, clumping factor A (ClfA), may be less important in bloodstream infections than was previously thought. This is because, unlike in iron-rich conditions, deletion of *clfA* produces no impairment of aggregation when exposed to iron-limited conditions. These findings may implicate iron-regulated proteins as an important factor in the haemostatic modulation ability of *S. aureus* in bloodstream infections and may form the basis of a novel mechanism of platelet activation by *S. aureus*.

A228

An unbiased screen for the gene set that defines Candida albicans cell wall mannans that are recognised by immune pattern recognition receptors

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Abstract

Candida albicans is one of the fungal pathogens that WHO recently reported as an urgent priority pathogen. New therapeutic approaches are urgently required to understand the ligands on the cell wall surface that activate and repress natural immunity. The fungal cell wall of *C. albicans* consists of an inner skeletal layer of β-(1,3)-glucan and chitin and an outer fibrillar layer of glycosylphosphatidylinositol (GPI)-anchored mannoproteins. These are modified with *O*-linked mannans that stabilise rod-like regions of proteins and *N*-linked mannans. Several pattern recognition receptors (PRRs) bind mannan in the outer cell wall of *C. albicans* and triggers immune responses. Of these dectin-2 is a major fungal PRR that is critically important for immune surveillance and is known to bind to mannan. However, the nature of the precise epitope for dectin-2 binding is poorly understood. We screened a transposon Piggy-Bac library for mutants that had increased and decreased binding of dectin-2 to identify the gene set that are critical to build and regulate the expression of the natural dectin-2 binding epitope. This identified both genes that are directly involved in mannosylation and a series of other genes that had not previously been implicated in immune recognition. This presentation will summarize progress of how this novel approach is changing our understanding of the genes that are required to build and regulate components of the fungal wall that are required for immune regulation and immunomodulation.

A229

An approach to characterising human intestinal colonisation with extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales in schistosomiasis-endemic regions of Malawi

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Abstract

The dissemination of extended-spectrum beta-lactamases (ESBLs) amongst Enterobacterales is a public health problem in Malawi, where ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are responsible for increased mortality in hospital patients with bloodstream infections. Outside of the hospital setting, ESBL-producing Enterobacterales commonly colonise human and animal intestines and are widely detected in Malawian wastewater and freshwater systems. Freshwater is also a key infection transmission source for parasitic diseases such as schistosomiasis, yet the effect of these microbiome-influencing parasites and associated environmental factors on AMR colonisation is not well understood.

By aligning AMR surveillance efforts with an ongoing study investigating novel hybrid schistosome (parasite) infections in Malawi, we examined faecal samples from 211 participants living close to excreta-contaminated freshwater sources in Mangochi and Nsanje Districts, southern Malawi. Bacteria recovered from faecal swabs were screened on CHROMagar ESBL. Resistant isolates underwent taxonomic classification via PCR amplification and analysis of 16S ribosomal RNA genes, before ESBL production was confirmed by the combination disc diffusion method. Parasite diagnostic data were generated within the schistosomiasis study by a combination of field-based and molecular methods.

We report high prevalence of intestinal colonisation with ESBL-producing Enterobacterales in southern Malawi (provisionally above 40%), with *E. coli* being the predominant species. We note a significant difference in the rates of ESBL-producing *K. pneumoniae* intestinal colonisation between our two study sites, with higher prevalence observed in Nsanje District ($p < 0.05$), and use regression analysis to investigate associations between schistosome infection, water contact habits, and colonisation with ESBL-producing Enterobacterales.

A230

Mitigating sepsis-related infection and inflammatory responses using novel phenolic derivatives

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Abstract

Sepsis is a life-threatening condition which impacts around 245,000 people in the UK every year. The condition is caused by a dysregulation of the host's response to a bacterial infection, triggering several interconnected systems leading to widespread inflammation and multi-organ failure. Therefore, in this study, we aimed to synthesise a multi-target drug (MTD) to combat the infection and its associated inflammation. The MTDs were synthesised based on a Naphthalimido lead compound, a polyphenolic derivative which has successfully shown antibacterial and anti-inflammatory characteristics. Compared to commercial antibiotic Gentamycin, in *vitro,* testing of the newly synthesised compounds has exhibited promising antimicrobial characteristics against Escherichia coli and Staphylococcus aureus. The Minimum Inhibitory Concentrations (MIC) of the two most prominent compounds, namely LB5 and LB15, were reported as 1.5µg/ml and 12.5µg/ml, respectively. Time-kill kinetics assays indicated the compounds were bactericidal rather than bacteriostatic, as the bacteria incubated (24 h, 37 °C) with LB5 and LB15 showed no viable growth on the total viable count (TVC) assay. Antioxidant investigations conducted using Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Oxygen Radical Absorbance Capacity (ORAC) assays demonstrated good antioxidant properties of both compounds compared to the Trolox equivalents. Further studies are underway for *in-vitro* cells (**RAW 264.7 murine macrophage cells).** The compounds' antibacterial and antioxidant properties underscore the promising potential of MTDs as a viable treatment for sepsis. Further investigation into specific drug targets and mechanisms of action will be conducted on lead compounds.

A231

Determining the mechanism of killing of lectin-like antibiotics

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Abstract

In recent years, there has been a global increase of antibiotic resistant infections. *Pseudomonas aeruginosa* is an important cause of hospital acquired infections that are associated with high levels of mortality. *P. aeruginosa* infections are becoming increasingly difficult to treat, with between 18 and 25% of clinical isolates showing multi-drug resistance and isolates found across the globe which are resistant to all or nearly all available antibiotics.

Bacteriocins, antimicrobial proteins deployed by bacteria for competition and colonisation, offer a potential tool to combat antibiotic resistance. The narrow-spectrum of bacteriocins, which will generally target bacteria closely related to the producing strain, could enable targeting of specific pathogenic bacteria without causing damage to the wider microbiome. There has been a range of bacteriocins discovered against *P. aeruginosa,* known as pyocins, with lectin-like pyocin L1 showing efficacy in infection models. However, we currently lack understanding of the mechanism of action of pycoin L1, which limits its development as a potential antibiotic therapy.

Our current aim is to identify the molecular target of pyocin L1 in *P. aeruginosa* using a range of genomic, biochemical and biophysical analyses. We are also further testing the activity of pyocin L1 against *P. aeruginosa* biofilms; and determining its ability to enhance the activity of other anti-pseudominal antibiotics.

A232

RamA -mediated regulation of intrinsic permeability in *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae (*Kp*) is a multidrug resistant pathogen which poses a threat to the public health worldwide. The intrinsic AraC-type regulator, RamA, plays a critical role in the regulation of multiple genes linked to membrane permeability in *Klebsiella pneumoniae*. Given that RamA overexpression is easily selected following antibiotic challenge, we sought to establish the parameters of RamA regulation on various permeability loci in *Klebsiella pneumoniae*. We applied a bioinformatics approach to mine for a marbox binding site with stringent sequence and location parameters against recognised permeability targets in *Klebsiella pneumoniae.* Promoter fusions were generated by cloning predicted binding sites into the promoter-less pKC26 plasmid and assessed for fluorescence levels in the presence and absence of RamA. Antibiotic susceptibilities were established according to the BSAC guidelines. Our bioinformatic analyses demonstrated a RamA-binding site, for, *oqxAB*, *mdtABC*, *macAB*, novel putative RND pump *hlyD*-3 and novel ABC pumps, KPN_00518 and KPN_00298. Gene expression showed upregulation of these genes in the presence of RamA where *oqxAB* (2.29- fold), *macAB* (1.33- fold), *mdtABC* (2.39- fold), *hlyD*_3 (2.2- fold), KPN_00518 (1.5- fold) and KPN_00298 (1.65- fold) respectively. Promoter fusion experiments indicated that the predicted marbox sequence is required for activation (1.5- 2- fold) in the presence of RamA. Specific efflux mutants demonstrated reductions in antibiotic susceptibility to tetracycline, tigecycline, chloramphenicol and ciprofloxacin.

Our results show that RamA is a key regulator of *Klebsiella pneumoniae* intrinsic permeability, via the activation of multiple efflux pumps using a recognised binding site.

A233

Characterising the Proteome of *Salmonella enterica* Outer Membrane Vesicles Under Infection-Relevant Conditions

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Abstract

Salmonella enterica is a zoonotic pathogen responsible for over 93 million human infections annually. *Salmonella* serovars cause disease ranging from gastroenteritis (e.g.: *S.* Typhimurium in humans, cattle, and pigs) to systemic disease (e.g.: *S.* Dublin and *S.* Choleraesuis in cattle and pigs, respectively, and occasionally humans). Like other Gram-negative bacteria, *Salmonella* produces outer membrane vesicles (OMVs) that play a role in pathogenesis by fusing to host cells to deliver virulence factors and manipulate the immune system. Protein packaging into OMVs differs between serovars and is influenced by environmental conditions. This research aims to characterise the OMV proteomes of *S*. Typhimurium, *S*. Dublin, and *S*. Choleraesuis when cultured in lysogeny broth (LB) and when exposed to intestinal levels of bile (1%) from cattle and pigs to identify serovar-specific differences that relate to the type of disease they cause. OMVs were purified from late logarithmic phase cultures using density gradient ultracentrifugation, visualised by SDS-PAGE, and quantified by nanoparticle tracking analysis. Periplasmic proteins were prepared at the same growth phase by cold-osmotic shock. Samples were analysed by data-independent acquisition mass spectrometry and initial observations indicate serovar and condition-specific differences in the OMV proteome and periplasm. An additional ~35 periplasmic proteins are packaged into OMVs in the presence of bile compared to LB. Furthermore, many cytoplasmic proteins were found in the bile OMVs indicating that bile may cause protein leakage into the periplasm. Comparisons to gene function and expression datasets will be made to understand the response of *Salmonella* to bile and its consequences.

A234

Phenotypic and Genotypic analysis of *Achromobacter* isolates misidentified as *Pseudomonas aeruginosa* in clinical trials for Cystic Fibrosis and Bronchiectasis therapeutics.

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Abstract

Achromobacter xylosoxidans (Ax) is intrinsically resistant to many antimicrobials and has been identified as an emerging pathogen of concern for people with Cystic Fibrosis (pwCF). Colonization with Ax has been associated with increased inflammation, more frequent exacerbations, and more severe lung disease.

Recently, we identified numerous isolates of Ax which had been misidentified as *Pseudomonas aeruginosa* (Pa) across two clinical trials. Interestingly, some Ax isolates were recovered when samples had previously cultured Pa suggesting potentially fluctuating dynamics between these pathogens. While diagnostics such as MALDI-TOF are used in clinical laboratories for first isolation, these approaches are not routinely embedded within trial settings. Furthermore, previous studies have highlighted that standard MALDI-TOF analysis is insufficient to distinguish between *Achromobacter* spp. therefore species of potential importance could go undetected. In this study, we performed both phenotypic and genotypic analysis from a collection of 52 isolates revealing high antimicrobial resistance to a panel of up to 12 antimicrobials, some of which were used as therapeutics in the trials. Whole genome sequencing revealed the phylogenetic relationships and information relating to hypermutation development, a key feature of chronic colonisation with Ax.

An understanding of phenotypic and genotypic diversity within isolates recovered from different respiratory infections are lacking. Ultimately, this work could help refine methodologies to prevent misidentification in these settings, which could mask microbiological treatment responses.

A235

Environmental and genetic regulation of Streptococcus pneumoniae galactose catabolic pathways

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Abstract

Efficient utilization of nutrients is crucial for microbial survival and virulence. It is not unusual that the same nutrient may be utilized by multiple catabolic pathways, indicating that the physical and chemical environments for induction as well as their functional roles may differ. Here, we studied the tagatose and the Leloir galactose catabolic pathways of the important human pathogen *Streptococcus pneumoniae,* determined the conditions important for induction of each pathway *in vitro*, and assessed their genetic regulation and *in vivo* expression. Our results show that while tagatose pathway induction occurs with as little as 0.5 mM galactose, the Leloir pathway requires 20 mM for a significant induction. While the absence of oxygen reduces the induction of both pathways, temperature has a differential impact on each pathway, increasing the induction of the tagatose pathway but not the Leloir pathway. In vitro, the Rgg144/SHP144 and Rgg1518/SHP1518 cell-cell communication systems are required for induction of the tagatose pathway. On the other hand, the Leloir pathway is repressed by Rgg144 while Rgg1518 has no impact. In vivo, the tagatose pathway is induced as early as 4 h post-infection, while the Leloir pathway induction was recorded at 32 h post-infection, and rgg1518 is required for the induction of the tagatose pathway. The results suggest that galactose catabolic pathways can be targeted to control microbial infections.

A236

In vitro evolution of *Klebsiella grimontii* to piperacillin-tazobactam resistance reveals identical and unique genomic changes between lineages compared to in-patient based evolution

Ellie Allman[1], Natasha Niethamer[1], Aakash Khanijau[2,3], Rachel Mcgalliard[2,3], Richard N. Goodman[1], Christopher M. Parry[1,3], Enitan D. Carrol[2,3], Adam P. Roberts[1]

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Abstract

Experimental evolution of pathogenic bacteria to antimicrobial resistance (AMR) has the potential to inform antibiotic therapy. However, there is limited understanding of how *in vitro* AMR evolution can replicate evolution of AMR within the human host. A trio of *Klebsiella grimontii* isolates cultured from a hospital in-patient with a recurrent bloodstream infection showed development of piperacillin-tazobactam (TZP) resistance over a four-month period due to a single nucleotide polymorphism (SNP) in the promoter of a chromosomal $bla_{OXY-6-4}$. To test if the same evolutionary pathways are followed in laboratory evolution compared to in-patient, the susceptible ancestor was exposed to sub-inhibitory concentrations of TZP in LB broth, followed by growth on TZP-supplemented LB agar. Resistant colonies were selected, and fitness, TZP susceptibility and genomes were compared to the ancestor and the *in vivo* evolved resistant isolate. In one *in vitro* evolved lineage, we observed the same $bla_{OXY-6-4}$ promoter SNP as seen in the *in vivo* evolved resistant strain which conferred high-level TZP resistance, however all other adaptive mutations were unique to either the *in vivo* or *in vitro* evolved lineages. The acquisition of TZP resistance did not confer any negative fitness consequences in the laboratory-evolved strains, in contrast to reduced fitness of the in-patient evolved strain. This study highlights different evolutionary trajectories in laboratory-based AMR evolution when compared to in-patient evolution and emphasises the need for comprehensive experimental design and cautious translation of findings to the clinic, particularly when interpreting fitness data from laboratory experiments.

A237

Repurposing drugs to target militarily relevant information

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Abstract

Inflammation can cause serious damage to cells/tissues in humans. This can happen for many reasons, for example: pathogenic infection, chemical agent exposure, blast injuries or other traumatic events. Our military medics might encounter inflammation caused by these threats. This project focuses on the potential dysregulated and pathologic inflammation bio-threat agents might cause. Pharmacies stock drugs that can produce anti-inflammatory effects and therefore could promote resolutions for pathologies. Here, we develop tools to measure inhibition of inflammation, the first step in repurposing these drugs.

An inflammation assay was developed where immune cells were cultured with immune stimuli. These immune cells could then be exposed to different concentrations of anti-inflammatory drugs that mirror human usage. Cell inflammation was read via titration of multiple inflammatory signalling molecules (cytokines). We have utilised state-of-the-art, fully automated assay technology for this purpose. This technology is being adapted for high containment (Containment Level-3) laboratory use. Using this developed assay, we hope to arm the medical community with knowledge of which anti-inflammatory drugs are likely to have effects on particular medical conditions.

BLOCK A

Session : Microbiota-Immune System and Vaccine Interplay (collaboration with British Society for Parasitology and Protistology UK)

A238

Commensal *Neisseria cinerea* outer membrane vesicles as a platform for the delivery of meningococcal and gonococcal antigens to the immune system

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Abstract

An affordable, accessible, and broadly protective vaccine is required to tackle the re-occurring bacterial meningococcal epidemics in Sub-Saharan Africa as well as an effective control of multi-drug resistant strains of gonococcus. Outer membrane vesicles (OMVs) secreted from Gram-negative bacteria represent an attractive platform for antigen delivery to the immune system and therefore for development of multi-component vaccines. In this study, we describe the generation of modified OMVs (mOMVs) from commensal biosafety-level 1 (BSL-1) *Neisseria cinerea* ATCC® 14685, which is phylogenetically close to the pathogenic bacteria Neisseria meningitidis and *Neisseria gonorrhoeae*. mOMVs were prepared from *N. cinerea* engineered to express heterologous antigens from *N. meningitidis* (factor H binding protein (fHbp) and Neisseria Heparin Binding Antigen (NHBA-2)) and from *N. gonorrhoeae* (NHBA-542). Mice immunised with the mOMVs, expressing various combinations of these antigens, produced antibodies against fHbp and NHBA. The work indicates that mOMV from *N. cinerea* can be used as a platform to induce immune responses against antigens involved in the protective immune response against meningococcal and gonococcal diseases. Furthermore, there is an indication that this immune response is dependent on mOMV concentration per dose regimen, as well as the inclusion of aluminium hydroxide adjuvant.

BLOCK A

Session : Microbes as sentinels and solutions in a changing world

A240

Engineering phenylalanine ammonia lyase to limit feedback inhibition by cinnamate and enhance biotransformation

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Abstract

Phenylalanine ammonia-lyase (PAL) is an enzyme with significant implications in biotechnology, particularly in the synthesis of phenols, antioxidants, and nutraceuticals. However, its practical application is constrained by feedback inhibition from its product, cinnamic acid, which impedes the forward reaction rate.

To tackle this issue, advanced enzyme engineering strategies have been employed. Both random and site-directed mutagenesis methodologies have been utilized to identify and isolate mutant enzymes that exhibit enhanced resistance to the inhibitory effects of cinnamic acid.

Through a systematic process involving high throughput screening and subsequent biochemical characterization, a thermotolerant and cinnamate-resistant mutant was identified. Among seven meticulously selected mutations, the T102E mutation stood out as the most promising. It demonstrated a substantial six-fold reduction in PAL's affinity for cinnamic acid and a two-fold increase in operational stability compared to the native PAL enzyme.

By immobilizing the enzyme on carbon nanotubes, its robustness and reusability were significantly improved. The immobilized mutant PAL was found to be more effective in deaminating phenylalanine present in protein hydrolysate than its non-immobilized counterpart.

Molecular dynamic simulations confirmed the increased tolerance to cinnamic acid, offering further insights into the underlying mechanisms. The findings not only broaden the understanding of PAL's sequence-function relationship but also pave the way for future advancements in enzyme engineering. The ultimate goal is the development of a highly tolerant and efficient version of PAL, expanding its potential applications in biotechnology.

A241

Elucidation of *Bacillus dicomae* sp. nov., spore structure and development of *Bacillus*-based formulation.

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Abstract

Endophytes are an endosymbiotic group of microorganisms that exist inside plant tissues without causing any negative impact. Bacillus species have the ability to produce spores, which gives them the ability to sustain harsh environmental conditions. As a result, their capacity to produce endospores ensures that they can withstand unfavorable environmental conditions, making them effective biocontrol agents. The objective of this study was to characterize the spore structure and develop a carrier-based formulation of the bacterial endophyte Bacillus sp. strain MHSD28 as a biocontrol agent. Powder formulation of three different carrier materials, which includes talcum powder, activated charcoal and sodium alginate were evaluated and a talc-based formulation was optimized for longer shelf life in terms of storage temperature and microbial concentration

A243

Use of a microbiome signature to determine health, productivity and welfare status in dairy cattle

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Abstract

Dairy products remain an important source of nutrition for people globally. As dairy cattle farming intensifies, there is a need to introduce innovative strategies to monitor the health, welfare and productivity of these animals. A growing number of studies highlight the use of gut microbiome signatures as diagnostic indicators for health conditions. To date, a large proportion of microbiome research in cattle has focused on 16S rRNA studies of the rumen, some of which have highlighted the association between prokaryotic taxa and certain health and productivity traits.

The current study sought to investigate the oral and faecal microbiomes of dairy cows and heifers around the time of calving. Pre- and post-calving oral and faecal samples were obtained from 150 animals on commercial UK dairy farms. Body condition, rumen fill and hock scores were assigned at each sampling timepoint and health and productivity data were collected during the lactation period. Shotgun metagenomic sequencing was employed to characterise microbial populations present in the samples and may provide an insight into the functionality of these communities. Further analysis will determine whether an association exists between these microbiome signatures and cattle health, welfare and production parameters.

Data from this project will expand the currently limited bovine oral and faecal microbiome knowledge base. This may be of practical significance given the relatively accessibility of these microbiomes compared to that of the rumen; the oral and faecal microbiomes could serve as important tools to predict and monitor the wellbeing and productivity of dairy cattle.

A244

The *Pectobacteriaceae* panCAZome and associations with host preference

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Abstract

Like all micro-organisms, plant pathogens depend on environmentally-derived nutrients and energy sources, such as complex carbohydrates. Niche-adapted microbes are expected to adapt to exploit environment-specific resources by developing appropriate metabolic and enzymatic capabilities for processing them. For instance, host-restricted plant pathogens might be expected to develop Carbohydrate-Active enZyme (CAZyme) repertoires targeting the liberation of sugars from complex molecules specific to their hosts. In this way, a sequenced pathogen's CAZyme complement might help predict its host range, or potential for host jumps.

Using software developed within our group we predict, catalogue and analyse the CAZyme complements of phytopathogen genomes from the *Pectobacteriaceae*, including *Pectobacterium* and *Dickeya* spp. (soft-rot pathogens), and *Brenneria* and *Lonsdalea* spp. (pathogens of woody plants). We identify diverse and distinctive CAZyme repertoires of these Enterobacterial plant pathogens, including coevolving sets of CAZymes that distinguish between taxa and that are potentially associated with host range. In particular, we identify CAZymes that appear to be specific to pathogens of woody hosts. These may be useful novel candidates for engineering towards more efficient industrial processes and achieving net zero targets, such as breakdown of recalcitrant woody lignocellulosic material in the production of biofuels.

A245

"Ecoligy" – Genomic & Phenotypic surveillance of faecal coliforms in the River Deben; what you count isn't what you get.

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Abstract

Faecal contamination of inland waterways is a hot topic both politically and scientifically. Freshwater ecosystems are under increasing pressure from human activity and climate change, creating one of the most widely recognised environmental challenges in the UK. In 2022 over 389,000 raw sewage discharges were reported. The Rivers Trust suggest that only 14% of UK rivers are in good ecological health. We collaborate with a 50-member citizen science group who perform traditional water quality testing assays including faecal coliform counts, nitrate and phosphate tests along the River Deben. This stretch of the Deben includes five wastewater / sewage treatment works and samples have been collected upstream, at the outflow and downstream of these facilities.

Coliform counting methods have remained largely unchanged for decades and rely on selective media and an indicator of glucuronidase activity, causing positive strains to appear blue. Determination of faecal coliform levels is therefore restricted to glucuronidase positive strains which excludes *E. coli* strains such as O157:H7 that lack this enzyme. We have established a workflow to culture isolates from Petrifilm coliform count plates onto MacConkey agar, which reveals more strains and taxonomic diversity than those which are counted. Nanopore sequencing reveals the taxonomy of these isolates along with detection of AMR genes and toxins. This analysis revealed the presence of 'non-coli' *Escherichia spp.* and *Klebsiella spp.* and >50% of strains carried plasmids. A curated library of 470 strains in 96-well format that facilitates rapid phenotypic screening for biofilm formation, antibiotic resistance and cytotoxicity has been constructed.

A246

AMR plasmid persistence during CRISPR-Cas9-directed removal can be overcome by toxin-antitoxin mitigation.

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Abstract

Antimicrobial resistance (AMR) genes are often encoded on mobile genetic elements, particularly plasmids. Removal of AMR plasmids is a promising approach of resensitising pathogens to antibiotics. We have previously shown that the genome editing tool CRISPR-Cas9, delivered by bacteria carrying the conjugative plasmid pKJK5::csg, leads to straightforward removal of a simple artificial AMR plasmid. However, it is unclear how well conjugative CRISPR-Cas is able to remove naturally occurring AMR plasmids.

Here, we show that multi-drug resistance conjugative plasmid RP4, naturally occurring in soil bacteria, is resistant to conjugative CRISPR-Cas9-directed removal, and that its *parABCDE* toxin-antitoxin operon is sufficient to achieve this effect. Mitigating toxin-antitoxin genes by supplementing them *in trans* on an expression vector can overcome this hurdle and successfully remove RP4. Likewise, adjusting our CRISPR-Cas9 delivery strategy by inducing target bacteria to carry pKJK5::csg before their exposure to RP4 successfully prevents RP4 colonisation of target bacteria. We conclude that the benefit of CRISPR-Cas9 in inter-plasmid competition is limited to the case where CRISPR-Cas9 is delivered to the target bacteria first, unless it is combined with toxin-antitoxin mitigation.

This work highlights the limitations of an active CRISPR-Cas immune system in inter-plasmid competition and shows how toxin-antitoxin-mediated target plasmid persistence can be circumvented to successfully cure antimicrobial resistance using CRISPR-Cas9-directed tools.

A247

The ecology of strain replacement in bacterial communities

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Abstract

Bacteria compete in two main ways: via nutrients (resource competition) and antibacterial mechanisms (interference competition). While both are important, we lack a general understanding of how they interact to define ecological success in bacterial communities such as the gut microbiome. Here, we address this with an eco-evolutionary model of bacterial competition. Our model predicts that invading strains – whether they possess mechanisms of interference competition or not – are unable to grow if a resident strain has depleted resources. This favours metabolic diversification, which coincidentally empowers interference competition. We experimentally confirm our predictions and use this principle to identify *Escherichia coli* strains that invade well and, if armed with natural antibacterial toxins, will displace multidrug-resistant clinical *E. coli* isolates. Recently, we showed that invasion into the gut microbiome is determined by nutrient overlap between the broader community and an incoming strain[1]. Therefore, we added up to 15 human gut symbiont species to our focal competitions between *E. coli* and showed that toxin-mediated strain displacement relies on metabolic differences not only between the two competing *E. coli* strains, but between the broader community and invading strains. This suggests efficient strain replacement relies on private nutrients specific to invading strains. We verified this prediction by supplementing an invading strain-specific nutrient, sorbitol, leading to successful toxin-mediated displacement of a target strain within a diverse community. Our work identifies general principles that shape the composition of bacterial communities and suggests ways to perform targeted replacement of problematic bacteria.

[1]Spragge* and Bakkeren* *et al. Science*. 2023.

A248

Long-read genome sequencing of *Escherichia coli* to augment an integrated AMR surveillance pilot in Uganda

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Abstract

Closer collaboration between One Health sectors is essential for improving our understanding and response to infectious diseases, and the associated emergence of antimicrobial resistance (AMR). This pilot project involves Uganda's Ministry of Health (MoH), the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) and the Ministry of Water and Environment (MWE) in Uganda to develop an integrated AMR surveillance dataset. However, the integration of surveillance data faces challenges due to diverse procedures, ethical and legal obligations, and differences in infrastructure across different sectors.

This project aims to create an integrated One Health AMR surveillance dataset by integrating existing AMR surveillance efforts across these institutions. It involves generating a total of 432 whole genome sequences of *Escherichia coli* using long-read sequencing on the Oxford Nanopore MinION platform. This high-resolution genomic data will augment an integrated and harmonised dataset of contextual data, enabling fine-scale understanding of AMR determinants and their distribution across One Health sectors.

Beyond creating a valuable microbiological resource, this pilot will also demonstrate the feasibility and challenges of developing an integrated One Health AMR surveillance system. It will test and evaluate how FAIR (Findable, Accessible, Interoperable and Reusable) principles can be operationalised. The system will capture and filter data, creating a shared resource for epidemiological analysis and interpretation. The integration and sharing of One Health data is expected to transform interdisciplinary collaboration and improve evidence-based decision-making, benefiting the health of humans, animals and the environment.

A249

Evolving potent toxin producers via bacterial competition

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Abstract

The world needs alternatives to conventional antibiotics. A promising option is to use bacteria against bacteria and develop biotherapeutic strains that produce specific toxins to target AMR pathogens. These exist in nature and can be engineered, but require optimisation for potency and fitness in an ecological context. Experimental evolution could allow potential biotherapeutics to evolve potency, while maintaining sufficient fitness.

However, there is a problem when trying to evolve improved toxin producers. In standard mixed culture, weak killers, that are immune to the toxin but do not carry the cost of better toxin production, benefit from clearance of the target strain and proliferate (“cheat”). Under such conditions, better toxin producers would be overgrown and not selected for. A spatially structured environment could be the solution, as toxin production benefits only the producer and leads to its enrichment.

We provide said spatial structure in microdroplets generated in a flow-focusing microfluidic device. Encapsulation of a synthetic microbial community allows high throughput screening of population dynamics and selection for potency. Outcomes of within-droplet competitions can be quantified by fluorescence microscopy and selected for success. We show that the droplet system is suitable for introducing higher degrees of spatial structure and for toxin producer enrichment over cycles of encapsulation and selection. In the future, this set-up could produce live biotherapeutic products with new or improved activities against a target strain.

A250

Surveillance and attribution of Scottish *E. coli* isolates

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Abstract

Escherichia coli is a promiscuous and ubiquitous bacterium associated with foodborne disease, as well as a useful sentinel for antimicrobial resistance, and the source of contamination of specific foods and environments from animal/human sources. Understanding the complex dynamics of *E. coli* infections and their attribution to specific hosts is pivotal in mitigating their impact on public health. This research focuses on the surveillance of *E. coli* across Scotland and is part of the PATH-SAFE Programme, which aims to develop a national surveillance programme for foodborne diseases and antimicrobial resistance.

Thanks to the tremendous support of consortium partners, we now have accumulated the genome sequences of over 4000 Scottish *E. coli* isolates from a diverse range of hosts and environments, including human, livestock, wild animals, and wastewater. We will present a preliminary analysis of the data, including phylogenetic structure and where related strains occur from different sources indicative of transmission, alongside AMR and virulence profiles across the population structure. A primary objective of the research was building of host attribution models based on previous approaches used for attribution of Salmonella isolates. Machine learning attribution models were generated based on single nucleotide polymorphisms, protein variants, and intergenic regions. Phylogenetic-based attribution models were also developed and compared against machine-learning approaches, as well as used to estimate the effects of missing phylogeny on the training datasets. The models will be applied to further understand transmission routes for *E. coli* in food and contribution to human disease, including bacteraemia and urinary tract infections.

A253

The importance of microbial ecosystems at the continental scale: tracking microbiological darkening and glacial melt in the Alps

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Abstract

25% of the global population is reliant on glaciers: melt is a reliable water source for irrigation as rainfall becomes more intermittent and unpredictable. The forecast for alpine glaciers is grim, with most not expected to persist beyond 2100. Ice and snow surface microbes contribute to the melting of glaciers by absorbing sunlight for photosynthesis, a phenomenon known as bioalbedo. Surface microbes are predicted to increase their growth as warming produces earlier and longer melt seasons, creating a feedback loop that further limits the lifespan of ice masses and brings forward the date at which their irrigative contribution must be replaced.

Tracking cryospheric microbes at scales sufficient to understand their contributions and responses to climate change is challenging, but is now possible. This study tracked blooms of microalgae and cyanobacteria in the Alps, using remote sensing and machine learning techniques in a novel hybrid approach, synthesising classical and leading edge techniques. A meta-omic study on the denizens of the ice surface from field samples demonstrates how community composition changes over the melt season, what organisms can survive and thrive in a sterilising environment, and how nutrient cycling occurs on the cryospheric surface.

Temporo-spatial evidence of blooming patterns and timing show the impact cryospheric microbiota have at continental scales as sources of albedo depression. This work highlights that microbiology is vital to large-scale terrestrial processes, and that detection and tracking of microbes in vast areas is becoming less challenging, allowing us to scale microscopic effects to the entire climate system.

A254

Methods for functional screening of fibre-based dietary prebiotics across gut-brain-axis targets.

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Abstract

The microbiota-gut-brain axis has emerged as a potential new therapeutic target for the effective treatment of both metabolic [1] and central nervous system disorders [2]. Diet is believed to be a major driver of the composition and function of the microbiota, with dietary fibres known to have beneficial effects, such as the release of metabolites with promising bioactive functionality [3]. In this study, three selected fibre-based prebiotics - fructooligosaccharides (FOS), galactooligosaccharides (GOS), and resistant maltodextrin were used to assess the impacts of bacterial-derived metabolites on microbiota-gut-brain axis signalling. Firstly, an APC-developed *in silico* pipeline was used to screen the genetic capacity of available probiotic bacteria to digest selected prebiotics, while producing neuroactive molecules based on curated metabolic pathways. Next, the ability of selected probiotic bacteria to use the prebiotic substrates as a carbohydrate source was assessed using a prebiotic activity score. They were ranked based on the growth in media with the fibres relative to the growth in media with dextrose. This guided the selection of the most promising probiotic/prebiotic combinations for further investigation based on metabolic potential. Cell-free supernatants of the combination of four selected probiotic bacteria and three prebiotics were subsequently investigated in *in vitro* assays to probe mechanisms of action with a focus on targets in the gut-brain axis. Overall, this *in silico* and *in vitro* approach allows for functional screening of symbiotic combinations with the strongest metabolic potential and brain health benefits.

[1] https://doi.org/10.1179/1476830513Y.0000000099

[2] https://doi.org/10.1152/physrev.00018.2018

[3] https://doi.org/10.1016/j.ebiom.2020.102968

A258

Biological tracking of nitrogen pollution in mangroves- biomonitoring using 'omics' based approach for mangrove restoration

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Abstract

The mangroves surrounding the coastal Bay of Bengal of the Northern Indian Ocean receives nitrogen inputs from multiple sources including anthropogenic activities with effects on structure and functioning of resident biological communities. The main objective of this study was to track the effect of dissolved inorganic nitrogen (DIN) pool influenced by anthropogenic nitrogen on resident bacterioplankton communities in Sundarbans, world's largest contiguous mangrove wetland and use the knowledge for mangrove restoration. Using 'omics' based high-throughput sequencing of bacterioplankton communities based on V3-V4 region of 16S rRNA and robust DIN pool quantification, a large number of stations representing from west to east of Sundarbans were classified as low DIN (>45µM) and high DIN (<40 µM) stations. Proteobacteria, Bacteroidetes, and Firmicutes were the dominant bacterioplankton phyla across all stations. Nitrogen-fixing groups including Nitrospirae and Planctomycetes were found to make up about 1% of the bacterioplankton communities. Abundances of Spirochaetes and Tenericutes showed a positive correlation with DIN. Pseudomonadales, Alteromonadales, and Desulfovibrionales varied distinctly in abundance between Low and High DIN stations. Predicted metagenomic profiles from taxonomically derived community structures indicated negative correlation of bacterial nitrate-nitrite reductase with prevalent DIN in High DIN stations but positive correlation in Low DIN stations. The identified high DIN stations also exhibited signs of mangroves. The new DIN approach coupled with 'omics'-based biomonitoring provides a new mechanistic classification of mangroves reeling from nitrogen pollution and is used for targeted restoration of mangroves in South Asia involving a nexus of science, society and policy framework.

A259

Measuring antibiotic-resistant pathogen abundance within the microbiota

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Abstract

The human microbiota is a complex community which plays an important role in health and disease. A healthy microbiota provides protection against colonization by foreign pathogens. However, in addition to the commensal species, pathogens such as carbapenem-resistant *Enterobacteriaceae* can be present, often asymptomatically at low abundance. Upon antibiotic treatment low-abundance antibiotic-resistant pathogens can overgrow, leading to hard-to-treat superinfections. Here we compare approaches to determine the abundance of potential pathogens, resistance genes, and commensal species within a model microbial community of human gut bacteria in vitro. Utilizing droplet digital PCR, metagenomic sequencing, and selective plating, we assess the ability to different approaches to detect resistant pathogens and measure changes in pathogen abundance following antibiotic treatment. Ultimately, detecting low-abundance resistant pathogens and identifying antibiotic-induced pathogen overgrowth will help current efforts to minimize the burden of drug-resistant infections.

A260

Microbial Hydrogen for Sustainable and Biocompatible Hydrogenation

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Abstract

Hydrogen based chemical reactions are powerful tools used to produce many everyday items; yet despite its value, >90% of H_2 used is derived from fossil fuels with significant environmental cost. Furthermore, chemical reactions which utilise H_2 often require harsh, energy intensive conditions which results in high CO_2 emissions to meet demand. This overwhelming reliance on fossil fuels cumulating in excessive greenhouse gas emissions is incompatible with ambitious 2050 climate goals, thus urgent green solutions are required for both aspects of H_2-based chemistry.

Despite the abundance of well characterised H_2-producing bacteria, the application of sustainable microbial H_2 for chemical synthesis remains underexplored. Furthermore, chemical hydrogenation catalysts have been shown to be compatible with living cells, opening doors to using living microorganisms to deliver sustainable H_2 within chemical processes. Here, I discuss our recent work in this area of *'biocompatible hydrogenation'*.

Beginning by outlining bio-H_2 production by uncharacterised microorganisms from the NCIMB culture collection, we then focus on biocompatible hydrogenation reactions using *Rhodospirillum rubrum*. Through a series of reaction screening experiments, we successfully achieve the hydrogenation of alkene containing substrates using microbial H_2 in combination with a biocompatible Pd catalyst.

Overall, our research demonstrates the potential of in situ biocompatible hydrogenations for sustainable synthesis, showing the first example of H_2 delivery for hydrogenation chemistry by a phototrophic microorganism. Furthermore, by demonstrating H_2 production by diverse bacteria from the NCIMB, we highlight the flexibility of bio-H_2 in chemical synthesis and how species' physiology can be chosen to suit specific industrial requirements.

A261

Considering colons, colibactin, and cancer

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Abstract

There is growing understanding of the relationship between microbes and carcinogenesis, as is illustrated by the carcinogenic action of the genotoxin colibactin, produced by many B2 *Escherichia coli* strains found within the human microbiota. Colibactin damages DNA and promotes chromosomal instability to directly induce tumorigenesis in colorectal cancer (CRC), the second-leading cause of global cancer-related deaths. Our results show that the D-enantiomer of the amino acid serine is capable of reducing colibactin production through transcriptional repression of the *clb* genes required for genotoxin biosynthesis. Data from experiments with colibactin-producing *E. coli* strains, CFT073 and Nissle 1917, suggest that D-Serine enters the cytoplasm of the bacterial cell via serine transporters such as CycA, DsdX, and YhaO, leading to downregulation of the *clbB* gene as observed via transcriptional GFP reporter assays. In an effort to decipher the interplay of D-Serine with the *clb* locus and in regulation of the colibactin operon, CFT073 and Nissle 1917 deletion strains lacking the Nitrogen regulatory protein C, *ntrC* (*glnG*) were created. Deletion of *glnG* results in increased levels of *clbB* transcription, with knockout mutants remaining susceptible to D-Serine-induced repression of colibactin production. Our research further elucidates the complex regulation of the colibactin locus and the mechanism of action by which D-Serine represses genotoxin production. As D-Serine shows the potential to reduce colibactin-associated CRCs, understanding of its repressive ability paves the road for a possible therapeutic candidate.

A262

Protein driven high-magnesium calcite mineralisation for the development of a biomimetic protective coating

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Abstract

Biomineralisation, the controlled formation of mineral structures by living organisms, is a fascinating process which offers exciting opportunities for innovations in materials science and biotechnology. In this study, we explore the biomineralization potential of proteins from *Pseudonocardia*, symbiotic bacteria found in the exoskeletons of *Acromyrmex* leaf-cutter ants, for the synthesis of high magnesium calcite (HMC) protective coatings. HMC, a difficult to (bio)synthetise carbonate (bio)mineral, is of particular interest due to its unique mechanical properties and potential applications in various industries.

Three candidate proteins were identified following a strategy that combined *Pseudonocardia* pan-genomic bioinformatics with ant *in situ* transcriptomics data. The candidate proteins have been engineered for heterologous expression, including identification of opportunities that allow incorporation of chemical handles hypothesized to accelerate the biomineralisation process.

FTIR spectroscopy has already been identified and tested as a method for discriminating between calcium carbonates, notably calcite and high magnesium calcite, using geological minerals for benchmarking. The characterisation and functionality of protein biomaterials and protein-driven biomineralisation was assayed via high-resolution imaging, spectroscopic and mechanical analyses including SEM, bioAFM, FTIR, EDS, and nanoscratching/indentation.

This research looks to advance our understanding of biomineralization processes in terrestrial environments, notably ants and their associated symbiotic microorganisms. Furthermore, the successful synthesis of HMC under fast and ambient conditions using protein scaffolds opens new sustainability avenues for biomimetic material synthesis and the development of advanced materials with tailored properties. As a carbonate, the material could also be deployed for carbon sequestration applications.

A263

The use of plant growth promoting endophytic and rhizosphere actinobacteria as a tool to improve the efficacy of soilless agriculture technology in the UAE

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Abstract

Hydroponics is one of the most important methods of modern agriculture to reduce the problem of water scarcity. The United Arab Emirates has established many hydroponic farms for their importance in agriculture and food self-sufficiency. This study aimed to find a new environmentally friendly method to improve the efficacy of hydroponics by using a mixture of actinobacteria that produces more than one type of plant growth regulators (PGR). Two different groups of actinobacteria producing PGR were isolated. The first group lives in the rhizosphere outside the roots, and the second group lives inside the plant roots. Both groups were tested for their ability to produce PGR. The study proved the ability of the actinobacteria that live inside and outside the roots to increase the production of green pepper and lettuce and give more statistically significant results than the treatment that did not include actinobacteria. The new finding in the current study was that the best treatment involved the actinobacteria that live inside the roots rather than those that live outside the roots. This study demonstrated a significant increase in the internal content of PGR in pepper and lettuce in the treatment that included actinobacteria that live inside the roots compared to the rest of the treatments, and this explains the increase in the yield. This study is the first to use an environmentally friendly method to increase the efficiency of hydroponics using actinobacteria that live inside the roots.

A264

Using endophytic actinobacteria for biological management of mango dieback disease in the UAE caused by *Lasiodiplodia theobromae*

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Abstract

From surface-disinfested mango roots, 27 streptomycete and 11 non-streptomycete actinobacteria were isolated. Their ability to manufacture chitinase and to impede the growth of *Lasiodiplodia theobromae*, the causative agent of mango dieback in the United Arab Emirates, was assessed. Streptomyces griseus was the most inhibiting isolate; it generated a comparatively large amount of chitinase and broke down the hyphae of *L. theobromae in vitro*, resulting in significant plasmolysis and cell wall destruction. *S. griseus* crude culture filtrate showed antifungal action and considerably (P<0.05) decreased the pathogen's germ-tube growth and spore germination. All samplings conducted up to eight weeks after inoculation revealed the antagonist inside the root, suggesting that the endophyte may have a home in the roots of healthy mango plants. Under greenhouse circumstances, *S. griseus* dramatically (P<0.05) decreased the severity of the dieback disease. These isolates colonized the mango root similarly to the chitinase-producing wild-type strain of *S. griseus*. Still, the endophytic *Streptomyces* sp. isolate could not produce measurable quantities of chitinase, lyse *L. theobromae* hyphae, or decrease dieback in the greenhouse tests. This study is the first to document the control of an endophytic chitinolytic actinobacterium in mango roots on a soil-borne plant pathogen.

A265

Improving cucumber tolerance to drought stress in the UAE with the use of endophytic actinobacteria

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Abstract

Several management techniques have been implemented in the United Arab Emirates (UAE) to assist crops in overcoming the negative consequences of drought. In the UAE, date pits (DP) are waste byproducts from date-processing businesses. For the first time, we offer a bacterization approach comprising endophytic actinobacteria with potential field uses, locally made DP powder (DPP; carrier), and cucumber seeds. This agro-biotechnological application aims to: (1) compare the growth promotion activities of endophytic actinobacteria using DPP carrier with other commercial carrier formulations; (2) evaluate the drought tolerance and growth performance of cucumber grown on DPP or other carriers under conditions of water scarcity; and (3) ascertain the role of actinobacteria, which produces the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase, in mitigating the negative effects of drought on cucumber. Our findings demonstrated that applying endophytic actinobacteria and DPP worked well to stimulate cucumber growth when it was irrigated with little water. It is abundantly evident that DPP, in its combination guarantees the root-colonizing actinobacteria effective colonization of seeds and roots. In addition, we observed that when DPP and endophytic actinobacteria were combined rather than actinobacteria alone, water consumption efficiency rose by a factor of twelve in the extreme water shortage regime (20 percent FC). This is the first to show how to use DPP+ actinobacteria, to encourage the growth of drought-stressed seedlings and increase plant production in an ecosystem known as water scarcity. The results will contribute to developing large-scale plans to exploit the UAE's underutilized nutrient-poor dry lands to grow low-water-demand horticulture plants.

A266

One Health genomic epidemiology of Escherichia coli and antimicrobial resistance within the national Malawian poultry supply chain

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Abstract

Understanding the complex epidemiology of WHO-priority pathogens such as *E. coli, Klebsiella* and their antibiotic resistance patterns relies on holistic One Health approaches. In this study we have sampled a hierarchical poultry community breeding structure including poultry, farmers, and their environment across the central region of Malawi. We generated antibiotic enriched metagenomes from a subset of 24 samples and combined this with long read sequences of 110 *E. coli* and *Klebsiella* isolates resistant to clinically critical antibiotics for humans; ciprofloxacin and ceftriaxone.

We will map microbial and resistance landscapes to evaluate the contribution of 'vertical' transmission of resistance from founder flocks at the apex, down to multipliers and small-scale farmers, compared to 'horizontal' introduction, along the supply chain. Facilitated by long read sequencing we can resolve antimicrobial resistance genes (ARGs), their context and genomic patterns of plasmids to identify potential sharing events between hosts and dynamic AMR. Through a combination of structured sampling, long-read whole genome and metagenomic sequencing, we aim to capture the prevalence and transmission of AMR to identify pathways of AMR spread and the potential zoonotic risk of these within a complex environment. To present for the first time the phenotypic and genomic characteristics of *E. coli, Klebsiella* and its domicile microbiome recovered from a 60-year-old community poultry breeding system in Malawi.

A267

Impact of prebiotics and probiotics on the functional microbial diversity of degraded soils reforested with Douglas fir shrubs

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Abstract

Many anthropogenic factors, such as high productivity agriculture and intensive forestry, contribute to soil degradation. The reforestation of these degraded soils is a sustainable method for reallocating forest ecosystem services such as wood production, carbon sequestration and the restoration of soil biodiversity. The application of products that stimulate microbial communities, such as prebiotics and probiotics, could facilitate the planting of young trees by stimulating their growth and development in these deteriorated soil conditions. This project is the result of a collaboration with two non-academic partners, Ecotree and Gaiago, the former a forest manager and the latter a manufacturer of soil revitalizing products. The aim of the research is to study the effects of prebiotic and probiotic treatments on the taxonomic and functional diversity of soil microorganisms and the consequences for the growth of newly planted trees in degraded plots. To achieve this, three plots with different occupancy histories (i.e. crop, heathland following clear-cutting, grassland) were replanted with Pseudotsuga menziesii (Douglas fir) and on each plot, ten trees were treated with prebiotics and probiotics and ten trees received water as a control treatment. In order to understand the underlying impact of these treatments on the soil close to the roots of the plants, enzymatic and metabarcoding approaches were carried out coupled with q-PCR targeting microbial taxonomic markers of the bacterial and fungal communities, as well as certain functional genes involved in the nitrogen cycle.

A268

Microbial inoculants increase the potential functionality of the root microbiome of young vines

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Abstract

In viticulture, dead or unproductive plants are replaced by new ones which take at least three to ten years to reach the full grape potential. While the addition of bacterial inoculants is mostly used to control pathogens that are mainly linked to grapevine trunk diseases, their growth-potential are rarely studied in viticulture. Similarly, the arbuscular mycorrhizal fungi (AMF) are symbionts having the capacity to enhance grapevine development but are mostly studied for their ability to increase resistance to environmental stressors in cultivated vines. Considering that the inoculation of microorganisms can either create or deplete microbial niches, thus triggering a potential microbial dysbiosis, the purpose of this project was to explore the effects of bacterial and AMF addition, in combination or individually, on the soil and root associated microbiome of young grapevines. Grafted *V. vinifera* L. cv. Cabernet Sauvignon (CS) scion on 1103 Paulsen were individually inoculated with AMF (*Rhizoglomus irregulare* and *Funneliformis mosseae*) or in combination with a consortium of two characterized plant growth-promoting rhizobacteria (PGPR: *Pseudomonas veronii* and *Pseudomonas brassicacearum*). After five months in greenhouse, the combination of both PGPR and AMF stimulated the root biomass and the abundance of potentially beneficial bacterial genera, compared with the untreated condition or single inoculum. In other hand, the abundance of fungal genera associated with grapevine diseases was reduced in the root endosphere. An increase in metabolic functionality was perceived in the case of combined inoculation of PGPR and AMF.

A270

Bisphenol S impacts growth and transcription of commensal gastrointestinal Bifidobacterium adolescentis

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Abstract

BACKGROUND: Recent studies have suggested links between several pesticides, food packaging chemicals, and food processing chemicals and changes in microbiome and chronic diseases. No systematic screening has been undertaken to assess the direct impact of these agri-food chemicals on specific human gastrointestinal microbiota, at concentrations relevant to human dietary exposure.

METHODS: We screened 57 representative gastrointestinal bacteria species in the presence of 30 widely used agri-food chemicals at 1µM. We further characterised a growth interaction between Bisphenol S (BPS) and *Bifidobacterium* by screening 31 *Bifidobacterium adolescentis* strains and 20 strains of other *Bifidobacterium* species. Whole genome sequencing of all strains and RNA-seq was conducted to better characterise the relationship of *B. adolescentis* exposed to BPS.

RESULTS: We observed 41% of agri-food chemicals impacted the growth of at least one screened bacterial species. Notably, bisphenol compounds were overrepresented. To better characterise this overrepresentation of bisphenols, further screening found that 16/31 (51%) of *B. adolescentis* and 4/20 (20%) of other *Bifidobacterium* species were growth impacted when exposed to BPS. Comparative genomics analysis correlated the observed growth phenotype in *B. adolescentis* with accessory genome components relating to the cell wall. Transcriptomics of *B. adolescentis* exposed to vehicle control versus BPA versus BPS identified BPS-specific transcriptional changes to metabolism and stress response, distinct from that of BPA.

DISCUSSION: Our results are the first to systematically characterise agri-food chemicals impacts on growth as a phenotype and also apply genomics and transcriptomics to better understand a xenobiotic-microbiota interaction. Further studies are needed to understand the impact of these findings on human health.

BLOCK A

Session : Small Talk: Mechanisms of sensing and signalling at the host-microbe and microbe-microbe interface

A273

Determining the role of prey-derived outer membrane vesicles in predation by *Bdellovibrio bacteriovorus.*

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Abstract

The predatory bacterium, *Bdellovibrio bacteriovorus*, has potential as a “living antibiotic” due to its capacity to invade and kill drug-resistant Gram-negative pathogens. Translation of this therapeutic from “bench-to-bedside” requires an in-depth understanding of predator-prey interactions, including how prey may escape predation. **Outer membrane vesicles** (OMVs) are known to mediate interaction between bacterial species, and we hypothesize they may act as decoys or carry cargo which affects the viability of *B. bacteriovorus.*

To investigate the potential role of prey-derived OMVs in predation, we purified and characterized OMVs from two important clinical pathogens, *Serratia marcescens* and *Klebsiella pneumoniae*. OMVs were visualised by transmission electron microscopy (TEM) and their protein content characterised by LC-MS/MS. Proteomic analysis identified both prey package hydrolytic enzymes with the potential to damage *B. bacteriovorus* in addition to numerous outer membrane proteins commonly found in OMVs.

Purified OMVs added to predation assays were found to have little direct effect on *B. bacteriovorus* viability. However, predators in the presence of additional OMVs were unable to reduce the CFU/ml of prey as effectively as controls in the absence of purified OMVs (approximately 10-fold difference at all timepoints tested). Additionally, fluorescence microscopy using mCherry tagged predators revealed slower rates of bdelloplast (predator inside prey) formation in the presence of OMVs compared to controls.

Our preliminary findings suggest that OMVs may act as decoys, slowing the rate of predation and associated reduction of prey viability. Future studies will utilise TEM and transcriptomic analysis of the “predatosome” to better characterise OMV-predator interactions.

A274

Extracellular ATP is a signaling molecule in bacteria

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Abstract

In animals and plants extracellular ATP (eATP) functions as signalling molecule and regulates the immune response. During inflammation intestinal bacteria are exposed to elevated eATP originating from the mucosa. Whether bacteria respond to eATP is unclear. Here we show that non-pathogenic *Escherichia coli* responds to eATP at physiologically relevant concentrations by modifying its transcriptional and metabolic landscape. The use of a promoter library showed that the response to eATP is time-, dose- and medium-dependent. Genes related to lipid, amino acid, or vitamin metabolism were regulated. Metabolomics showed that eATP triggers the enrichment of molecules with bioactive properties on the host or bacteria. Combined genome-scale modelling highlighted the global metabolic modifications. Moreover, eATP altered the sensitivity to antibiotics and antimicrobial peptides. Finally, in pathogens eATP controls the expression of virulence and fitness factors. Our results indicate that eATP is a signalling molecule in prokaryotes which regulates physiology, antimicrobial resistance, and virulence.

A275

Specific bacterial odours serve as a nutrient code for *C. elegans*.

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Abstract

Secondary metabolites produced by bacteria enable them to thrive and deal with their neighbours. Recent studies have shown that bacterial odours can facilitate inter-kingdom interactions as well. Previous work from the lab has shown that 1-undecene, an odour produced by *Pseudomonas aeruginosa,* can induce aversion in *C. elegans*, a bacterivorous worm. We showed that 1-undecene can regulate immunity in *C. elegans*. We asked whether bacterial odours could also influence host dietary behaviour. To understand this, we studied olfactory bases of interaction between *C. elegans* and CeMbio, worms' natural microbiome. Using behavioural assays, we found that the worms are attracted to 3 bacteria in an olfaction-dependent manner. We found that the preferred CeMbio bacteria are sensed via the AWA and AWC pair of odour sensory neurons of *C. elegans*. Using SPME-GC/MS analysis, we found that preferred CeMbio bacteria share a common volatile. This volatile is a known attractant for *C. elegans* and is derived from catabolism of an amino acid in bacteria. We showed that the abundance of this volatile can be increased by supplementing the bacteria with this amino acid, which in turn enhances the attraction of worms to the bacteria. Finally, we showed that the robust response to this odour is retained in the wild isolates of *Caenorhabditis sp.* emphasizing its ecological relevance. Altogether, our findings suggest usage of an olfactory code to identify an amino acid-enriched diet by *C. elegans* in its habitat.

A276

Gut colonization of the sulphidogenic Bilophila wadsworthia under a high-fat diet

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Abstract

High-fat diets alter the gut microbiota composition and stimulate the proliferation of the sulfidogenic bacterium *Bilophila wadsworthia* (*Bw*). *Bw* expansion is linked to gut inflammation and dysfunction of the intestinal barrier and bile acid metabolism. The genetic basis for its colonization in the gut remains largely unknown.

In this study, we used a genome-wide transposon mutagenesis approach for *Bw*, TraDIS-Xpress, to identify genes essential for gut colonization of mice under a high-fat diet with or without a simplified humanized microbial consortium (SIHUMI). The effect of the microbiota on host health was also determined. Compared to *Bw* alone, the combination of *Bw* with SIHUMI caused a lower weight increase, higher gut permeability and abundance of the pro-inflammatory cytokines IL-1a and IFN-G. Comparison of the mutants present in culture, against the mutants in the gut, revealed that 82 genes were not-essential in culture but beneficial for gut colonization. These included genes for respiration and microcompartment formation, which allow *Bw* to efficiently respire taurine and isethionate. A higher number of genes was required by *Bw* for gut colonization when together with the SIHUMI consortia as compared to monoculture, including the synthesis of nucleotides and histidine.

Our results suggest that *Bw* uses microcompartments for competitive metabolism that allows it to thrive in the gut, similar to enteric pathogens. The exacerbated detrimental effect of Bw with SIHUMI, suggests that the microbial composition plays a key role in the modulation of the activity of this pathobiont.

A277

Characterization of AI-2 Signaling and Spoilage Properties in *Pseudomonas* from Meat Sources

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Abstract

Quorum Sensing (QS) constitutes a sophisticated bacterial communication system, allowing organisms to sense their population density using autoinducer molecules. While Gram-negative bacteria rely on autoinducer-1 (AHL), both Gram-positive and Gram-negative counterparts utilize autoinducer-2 (AI-2) to regulate various mechanisms. Exploring AI-2 signaling in spoilage-related microorganisms, particularly examining AI-2 responsiveness in *Pseudomonas fragi* devoid of AHL production. In this study, we aimed to conduct the relation between spoilage properties like motility, biofilm, proteolytic activity, and AI-2 production. Isolates (n=83) from beef and minced meat samples were characterized, identifying 15 putative *P. fragi* and 57 closely related *P. bubulae* via *rpo*D primers. All isolates exhibited robust motility (swarming, swimming, and twitching). Biofilm formation assays indicated 37 isolates with significant biofilm production, quantified using a well-plate spectrophotometer. Assessment of AI-2 production through luminescence via *Vibrio harveyi* biosensors revealed activity in 69 isolates. Future investigations aim to elucidate the impact of AI-2 production gene deletions on these divergent behaviors. This study illuminates how AI-2 signaling works in *Pseudomonas* species found in meat, revealing how communication influences spoilage characteristics. Understanding AI-2's impact on spoilage-related mechanisms provides valuable insights into managing microbial spoilage in meat-based environments.

A278

Effect of inoculum size and antibiotic pressure on the interplay between nasal staphylococcal commensals and exogenous MRSA

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Abstract

Staphylococcus aureus is known to colonise the nasal cavity of around 30% of the adult population, along with other commonly isolated bacterial species such as Corynebacterium spp. and coagulase negative staphylococci. This project aimed to assess (i) the role of inoculum size and nasal colonisation by exogenous MRSA; (ii) effect of antibiotic exposure on the colonisation ability of MRSA.

Nasal swab from a healthy individual was resuscitated and used as source of nasal staphylococci. Overnight broth cultures of nasal commensals were co-cultured with low (104 CFU/mL) and high (107 CFU/mL) clinical MRSA isolate to assess survival of both species. The impact of antibiotics including mupirocin, used for decolonising MRSA positive individuals, was also assessed.

Growth of exogenous MRSA was reduced in the presence of nasal staphylococci. Growth of MRSA at an inoculum size of 104 CFU/mL was significantly reduced compared with 107 CFU/mL. In the presence of mupirocin, decline of both commensal and MRSA population was observed. Additionally, use of sub-inhibitory concentrations of antibiotic that MRSA was resistant to led to loss of nasal staphylococci after 26 hours.

Nasal commensals of healthy individuals can prevent colonisation with invading exogenous MRSA isolates and is dependent on the inoculum size. Antibiotic exposure alters the interplay between MRSA and nasal commensals. Treatment with mupirocin, likely makes a healthy individual vulnerable to potential colonisation by exogenous MRSA in future.

A279

Impact of metabolites released by pathogenic microbes on wound healing

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Abstract

Opportunistic pathogens such as *Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa), and *Candida tropicalis* (Ct) commonly co-inhabit chronic wounds. The secretome of these pathogens may significantly contribute to delayed wound healing.

Clinical isolates of Pa, Sa, and Ct, obtained from diabetic foot ulcers, were cultured individually or in combination in Davis & Mingioli Medium A for 48 hours. From harvested supernatant, metabolites were extracted, and untargeted metabolomics performed using ESI-LC-MS. Using human fibroblast cells (HFCs), cell proliferation, trypan blue staining, cytotoxicity and cell migration assays were carried out.

A total of 1052 metabolites were identified in the individual cultures of Pa, Sa and Ct, while co-cultures of Pa-Sa and Pa-Ct showed 315 and 273 metabolites, respectively. A dose-dependent response was noted in cytotoxicity assessment; however, metabolites from co-cultures exhibited more cytotoxicity than those from mono-cultures. A notable decrease in proliferation was observed in metabolite-treated group when compared to the control group. Cell migration revealed a reduction in migration for HFCs treated with metabolites with metabolites from co-cultures exhibiting a substantial decrease in migration.

Our observations highlight the significant anti-migratory and cytotoxic properties inherent in pathogen-secreted metabolites. Virulence factors secreted by pathogenic microbes play a pivotal role in impeding the wound healing process by inhibiting inflammatory response, suppressing cell proliferation, and hindering re-epithelialization. Our investigation shows that the microbial secretome exerts a discernible impact on skin cells and slows the closure of wounds, consequently resulting in delayed healing of ulcerations

A280

Understanding the Role of Actomyosin Regulators During *P. aeruginosa* Infection in *C. elegans* Intestinal Epithelium

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Abstract

During pathogenic infections, interactions between the host and the pathogen can occur either directly through physical adherence or indirectly through secreted molecules. This dynamic determines the trajectory of the pathogenesis. The host employs various strategies to counteract the infection, and the structural integrity of tissues serves as the primary mechanical barrier to pathogen assaults. One notable example is the epithelial cells that line various organ systems; their shape plays a pivotal role in defense against pathogens. Any disruption to the epithelial lining triggers the cell's autonomous immunity and leads to the clearance of the pathogen.

The *C. elegans* intestinal epithelium, lacking specialized immune cells, provides an intriguing model system to investigate the role of epithelial cells in pathogenesis. *P. aeruginosa*, a pathogen known for affecting human epithelial linings, behaves differently in *C. elegans*, where it acts as an extracellular pathogen residing in the lumen. Our study focused on understanding the impact of *P. aeruginosa* infection on the architecture of the intestinal epithelium. We found that *P. aeruginosa* infection causes deformation in the *C. elegans* intestine, particularly affecting all apical regions of the tissue. Since the cell cortex is the fundamental component of the epithelial support structure, we conducted a candidate RNAi to identify key contributors to this deformation. We identified that GTPase CDC-42 is an upstream regulator inducing intestinal deformation following infection. Our work sheds light on the crucial physiological role of tissue architecture in the context of pathogenesis and provides a foundation for a deeper understanding of the underlying mechanisms.

A282

Unraveling the molecular basis of *B. thetaiotaomicron* induced anti-inflammatory responses

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Abstract

Intestinal homeostasis is achieved through a dynamic interplay between the resident microbiota and the intestinal immune system. Specific microbiota members, such as the prominent gut symbiont *Bacteroides thetaiotaomicron*, coordinate this homeostasis through production of specific molecules that promote anti-inflammatory responses. However, the molecular mechanisms that underlie these beneficial interactions are poorly defined. Using an *in vitro* model system we found that *B. thetaiotaomicron* elicits production of anti-inflammatory cytokines, like IL-10, in a TLR2-dependent manner in response to outer membrane vesicles (OMVs). Using a transposon mutagenesis library, we identified that gene *BT1160*, predicted to encode subunit A of the Na^+-transporting NADH ubiquinone oxidoreductase (NQR), is critical for *B. thetaiotaomicron*-mediated induction of IL-10. Deletion of this gene, the entire NQR complex (*BT1155-BT1160)* or all but gene *BT1160* (*BT1155-BT1159*) recapitulated these findings, demonstrating a key role of the NQR complex in the modulation of host immune responses and establishing its necessity for *B. thetaiotaomicron* to induce IL-10. Disruption of the NQR complex impaired OMV biogenesis, and normalization of the amount of OMVs produced by *nqr* mutants to that of the wild-type strain restored IL-10 induction by *B. thetaiotaomicron*. These data mechanistically link the role of the NQR complex in OMV biogenesis and underscore the requirement for coordinated OMV generation in immune modulation by *B. thetaiotaomicron*. Future work will investigate its role in the coordination of anti-inflammatory responses *in vivo*. Overall, these findings suggest that the *B. thetaiotaomicron* NQR complex promotes host-bacterial mutualism by favoring an anti-inflammatory intestinal milieu.

A283

Characterisation of the genetic determinants for R. hominis flagellin-host interactions

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Abstract

Roseburia hominis is an abundant constituent of the human gut microbiome and a member of the *Lachnospiraceae* bacterial family. Its ability to produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate has been associated with the modulation of gut microbial ecology and host energy homeostasis. We have recently shown that *R. hominis* uniquely produces “silent” flagellins that can bind to host toll-like receptor 5 (TLR5) without initiating a pro-inflammatory response. This suggests that these organisms can actively modulate their interaction with the host immune system, challenging our current understanding of flagellin-TLR5 interactions. We now seek to understand the genetic basis for silent and stimulatory flagellins and the mechanisms for their interaction with the host. To achieve this we systematically identified the requirements for successful DNA transfer to *R. hominis*, resulting in the first genetic system for this non-model organism. We identified four restriction-modification defence systems in *R. hominis* and characterised the methyltransferases and their subunits responsible for protecting its own DNA. Next, we developed an *in vitro* methylation strategy that was applied to a series of *E. coli-Lachnospiraceae* shuttle vectors, enabling DNA transfer and uptake at high efficiencies. We then constructed knock-out vectors to sequentially remove each flagellin gene from the chromosome and characterised mutant impact on *R. hominis in vitro* growth kinetics, substrate utilisation, and motility.

A285

Human CEACAM1 is targeted by a Streptococcus pyogenes adhesin implicated in puerperal sepsis pathogenesis

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Abstract

Puerperal sepsis, or child-bed fever, is a life-threatening bacterial infection most commonly caused by *Streptococcus pyogenes.* While outbreaks have been detailed in classical epidemics, puerperal sepsis remains a global health problem today. Repeated epidemiological studies have shown that the *S. pyogenes* protein R28 is over-represented in outbreaks of puerperal sepsis. Despite this, little is understood about the disease mechanisms, or the role of R28 in puerperal sepsis. Here, we identify the host receptor of R28 as human CEACAM1 and show this interaction to be highly specific through direct binding assays. High-resolution structural analysis revealed that a domain of R28 with an IgI3-like fold binds to the N-terminal of CEACAM1. Through biochemical investigation, we identified the interacting residues at the binding interface and determined that the interaction was highly human specific. Next, we showed this interaction promotes adhesion of *S. pyogenes* to human cervical cells through an epithelial adhesion assay. Moreover, we used an in vitro scratch assay as a measure of wound healing and showed the interaction suppresses epithelial wound repair. Finally, using an ex-vivo cervical explant model, we show this interaction subverts host innate immune response by quantification of protein level production of cytokines and chemokines. Taken together, these findings present a single adhesin-receptor interaction as responsible for driving pathogenesis of bacterial sepsis via distinct pathological outcomes. Here, we provide molecular insights and mechanistic information of one of the most important infectious diseases in medical history.

A286

Insights from Microbiota Metabolites: Deciphering Indole's Role in Interspecies Signalling and Its Impact on Salmonella Physiology

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Abstract

Salmonella are important foodborne pathogens which cause a spectrum of disease in humans ranging from gastroenteritis to Typhoid fever. Motility is essential in physiology and contributes to competitive advantage in finding a niche within the gut through chemotaxis. Bacteria sense and respond to stimuli through a mechanism called two-component systems. Colonisation resistance is a phenomenon whereby the innate intestinal microbiota repel invading pathogens from establishing a niche and causing disease. The small molecule indole, which is produced by members of the microbiota, has been shown to regulate basic physiology, virulence, and biofilm formation in a number of pathogenic bacteria, but does it affect motility in *Salmonella* and how is it sensed?

A287

Elucidation of novel human receptors for *Pseudomonas aeruginosa* for the design of effective antimicrobial therapies

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Abstract

Pseudomonas aeruginosa is a highly antimicrobial-resistant pathogen that causes difficult-to-treat acute and chronic infections, due to its high host adaptability. New antibiotic therapies against *P. aeruginosa* are urgently needed. Elucidation of host receptors for *P. aeruginosa* is critical for the design of innovative treatments against its infections. We have identified, for the first time, the role of protein disulfide isomerases (PDI) A1 and PDIA3 in *P. aeruginosa* attachment to epithelial cells using a novel unbiased 2D proteomic approach. Treatment of human bronchial epithelial cells (16HBE14o^{-}) with the PDI inhibitor, LOC14, showed a dose-dependent decrease in *P. aeruginosa* attachment to these cell lines (p=0.0188). *P. aeruginosa* attachment to HEK293T cells overexpressing PDIA1 and PDIA3 was higher than the control (empty plasmid); (p= 0.0360, 0.0132, respectively). Bacterial attachment to CRISPR cell lines A549 *pdia3*$^{-/-}$ was lower than to A549 cells (p= 0.0344) while attachment to A549 *pdia3*$^{-/-}$ transfected with a plasmid containing *pdia3* restored the attachment levels. Confocal microscopy suggested a co-localization of *P. aeruginosa* with the PDIs on the human cell surface. Finally, the *in silico* superposition of human PDI structural models with *P. aeruginosa* PDIs suggested a possible *P. aeruginosa* hijacking of host PDIs. Our study enables a better understanding of *P. aeruginosa* interaction with the host, opening the possibility of understanding a critical pathway for *P. aeruginosa* interaction with the human cells that might lead to the design of novel antimicrobial therapies or the use of currently available drugs that target human disulfide isomerases.

A288

Klebsiella pnuemoniae-induced sepsis causes excessive neutrophilic inflammation and worsens lung injury during loss of STAT1 regulation

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Abstract

Klebsiella pneumoniae (KP) are Gram-negative bacteria of public health concern due to their capacity to acquire antibiotic resistance and cause deadly infections. STAT1 is a master transcription factor activated through IFN signaling and helps defend against systemic spread of acute KP-intrapulmonary infection. We used *Stat1*$^{-/-}$ mice to study the impact of loss of IFN signaling on KP-induced sepsis.

Wild-type (WT), *Stat1*$^{-/-}$, and myeloid cell-specific STAT1-deficient (LysM$^{Cre/Wt}$;*Stat1*$^{fl/fl}$) mice were intratracheally inoculated with a KP clinical respiratory isolate (10^3 CFU) belonging to the hypervirulent K1 serotype. Bulk-RNA seq showed that *Stat1*$^{-/-}$ mice exhibited an exaggerated neutrophil signature in the lungs but showed no significant difference in lung CFU 24h post-infection. By 48h, *Stat1*$^{-/-}$ mice exhibited significantly increased lung CFU and extrapulmonary dissemination to the liver and kidney, whereas WT mice showed reduced KP extrapulmonary burden. By 72h, *Stat1*$^{-/-}$ mice displayed increased BAL neutrophil counts and free NE activity compared to WT mice. LysM$^{Cre/Wt}$;*Stat1*$^{fl/fl}$ mice demonstrated no significant difference in lung CFU and extrapulmonary sites compared to WT littermates at 48h post-infection. By scRNA seq, KP-infected lungs of *Stat1*$^{-/-}$ mice displayed heightened CD4^{+}T-cells at 24h. Flow cytometry analysis confirmed that IL-17-producing CD4^{+}T-cells were increased in the lungs of *Stat1*$^{-/-}$ mice at 24h post-infection. Blocking global IL17-signaling led to increased KP proliferation and dissemination, while CD4^{+}T-cell depletion reduced KP abundance in the lungs of *Stat1*$^{-/-}$ mice.

Our findings suggest STAT1 employs myeloid cell-extrinsic mechanisms to regulate neutrophil responses and protect against invasive KP by restricting non-specific CD4^{+}T-cell activation in the lung.

A289

Biological diversification of chemical signalling systems reveals distinct evolutionary patterns in keystone pathogens

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Abstract

Transcriptional regulators enable microbes to respond to external cues, presenting a tuneable response system involved in antimicrobial resistance, nutrient availability, metabolic reprogramming, quorum sensing signalling and cell-cell communication, efflux, and biotransformation. Recently, two families of transcriptional regulator have emerged with key roles in cell-cell communication and the shaping of microbial community dynamics. Acting as receivers and transducers for distinct chemical languages, members of the LysR- and LuxR- families of transcriptional regulators are now known to play key roles in the pathophysiology of infection. However, many of the proteins in these families remain uncharacterised and therefore it is important that their evolutionary trajectory is understood to uncover the functionality of this hidden 'control-ome'.

Comparative genomics revealed a wide distribution of LysR-type transcriptional regulators across *Pseudomonas aeruginosa*, with core LTTRs present in >90 % of the genomes and accessory LTTRs present in <2 %. PqsR, the receptor for the Pseudomonas quinolone signal (PQS) was found to be amongst the most variable in the dataset. Complementation of the PAO1 *pqsR*- mutant using representative variant PqsR sequences suggests a degree of structural promiscuity within the most variable of LTTRs. Comparative genomics also revealed that this promiscuity of diversification is seen in the LuxR-type transcriptional regulators. Best known as the receptor for the classical quorum sensing acyl homoserine lactone signal, photopyrones have recently emerged as a new chemical language operating through the LuxR system. We show photopyrones and their analogues have anti-biofilm and negative growth impacts on several ESKAPEE and other opportunistic pathogens.

A290

Interaction of *Fusobacterium nucleatum* subspecies with cancer cells leads to differential cytokine secretion

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Abstract

A growing body of research has shown an association of certain oral bacteria with colorectal cancer (CRC). Of particular interest is the oral anaerobic bacterium *Fusobacterium nucleatum*, which has been found to be enriched in CRC tissues compared to controls. Five subspecies of *F. nucleatum* have been described and a growing body of evidence suggests that that they have varying pathogenic potential.

This study investigated the impact of adhesion and invasion of three subspecies of *F. nucleatum*: *ssp. animalis*, *ssp. nucleatum* and *ssp. polymorphum* on colorectal cancer cells (CaCo-2), measuring the release of inflammatory mediators using the Olink target 48 panel.

A total of 3 cytokines were significantly differentially expressed out of 45 measured biomarkers, of which two (CXCL10 and IL-17C) were highly expressed in the *F. nucleatum* subspecies compared to a control. Whilst CXCL10 showed an increase in all subspecies, only subspecies *nucleatum* and *polymorphum* led to higher expression of IL-17C, which has been shown previously to aid in tumour promotion.

This study uncovered subspecies-specific differences in host-cell cytokine and chemokine secretion, demonstrating the complex interplay of inflammatory mediators, host factors, pathogens and their characteristics in the tumour environment.

Understanding the differences between *F. nucleatum* subspecies in host-pathogen interactions can uncover specific mechanisms which are key to their role in CRC.

A291

Co-zorbs: Motile, multispecies biofilms that aid transport of pathogenic bacteria

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Abstract

Microbial communities form spatially organized biofilms in which species adhere, multiply, and form microcolonies held in matrices of extracellular polymeric substances. Although biofilms have been thought to be stationary, *Flavobacterium johnsoniae* forms a unique spherical biofilm called a "zorb," which is propelled by its base cells. Here we report the first multispecies motile biofilms, designated co-zorbs, in which *F. johnsoniae* encapsulates and localizes other bacterial species within the core of the zorb. The spatial organization and formation kinetics of co-zorbs are cell-density dependent and occur only with metabolically active cells. Several bacterial species form co-zorbs with *F. johnsoniae*, including non-motile MRSA. Co-zorbs transport and disperse non-motile MRSA and other bacteria, underscoring the potential implications for co-zorbs in infectious disease. The discovery of co-zorbs expands our understanding of the complex interactions in microbial communities, challenges the perception of multi-species biofilms as static structures, and invites further exploration of the ecological and biomedical significance of co-zorbs.

A292

Oxidative stress constrains invasion of quorum-sensing defective mutants in evolving *Pseudomonas aeruginosa* populations

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that causes damaging chronic respiratory infections. During chronic infection *P. aeruginosa* populations undergo a suite of characteristic genomic adaptations to the lung environment, including loss of quorum sensing. However, what selective factors within the lung environment drive this evolutionary path remain poorly understood. Here, we used evolution experiments (>250 generations) and population genomics to test how lung environmental factors associated with inflammation, specifically oxidative stress and availability of free amino acids, drive the evolution of *P. aeruginosa* quorum sensing. We report that high levels of oxidative stress and availability of amino acids limited fixation of evolved quorum sensing negative (QS-) mutants. We then used population genome sequencing to determine the genetic basis of these contrasting evolutionary dynamics. Together our findings suggest that variation in the host environment arising from different levels of inflammation could drive differences in the evolutionary trajectory of *P. aeruginosa* populations with possible consequences for virulence of infections.

A293

Salt's signature in the human gut: how the Bacteroides/Prevotella ratio could reveal your sodium secrets

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Abstract

From ancient times, humans have sought salt, especially for preserving, flavouring, and texturing food. However, excessive consumption of sodium, mostly from salt, can lead to about 2.5 million deaths yearly. Excess sodium intake has been linked to various diseases, particularly cardiovascular diseases. There has been increased interest in the gut microbiome and the perturbation of these microbes has been linked to several health conditions. Recently, research efforts have been geared towards the interaction within these microbial communities and the identification of specific signatures in various physiological states and conditions. However, there is still a paucity of information on dietary sodium intake and its relationship with gut microbiota. Therefore, this study aims to elucidate the relationship between dietary sodium intake and the human gut microbiota. Here, using food consumption and metagenomic data, we investigate sodium intake and alterations of the human gut microbiota. We also used functional predictive tools to reconstruct pathways relevant to sodium reabsorption. We found that compared to low sodium diet (LSD), high sodium diet (HSD) altered the gut microbiota composition with a significant reduction in *Bacteroides* and an inverse increase in *Prevotella*. However, there is no distinct Firmicutes/Bacteroidetes ratio between the two groups. Since it is currently difficult to confidently associate the F/B ratio with what is considered healthy (e.g low sodium) or unhealthy (e.g high sodium), we suggest that the use of a genus-based ratio such as *Bacteroides/Prevotella* (B/P) ratio may be beneficial for the application of microbiome studies in health.

A294

Look who's talking: Can bacteriophages influence the arbitrium systems of neighbouring phages?

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Abstract

Bacteriophages are the most abundant biological entities on the planet. Many phages employ two lifecycles upon infection: lytic, where they lyse cells to release new phage particles, or lysogenic, where they integrate into the bacterial genome. SPβ-like phages that infect Bacillus utilise a communication system to coordinate their lysis/lysogeny decision (Erez et al, Nature 2017). Effectively, these phages employ quorum sensing to switch between lytic and lysogenic replication depending on the concentration of the “Arbitrium” signal molecules they produce (Erez et al, Nature 2017; Bruce et al ; Brady et al; Aframian et al). Crucially, closely related phages often produce different signal molecules, and anecdotal evidence suggests that phage only sense their own signal. However, recent data from our lab show that at least some SPβ-like phages can cross-talk. Systematic studies are lacking that explore cross-talk between SPβ-like phages. I will present experiments that investigate variation in the specificity / promiscuity of different signal receptors carried by diverse SPβ-like phages. Furthermore, I am analysing the structures of these receptors to identify the molecular determinants of signal specificity and to understand how novel specificities may evolve in these phages.

A295

Exploring the impact of the respiratory tract microbiome on epithelial integrity in chronic obstructive pulmonary disease (COPD) – do commensals vs. pathogens have differential influences on tight junction formation and maintenance?

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Abstract

There is a strong association between chronic obstructive pulmonary disease (COPD) and bacterial infection, with 50% of disease exacerbations being directly linked with infection by bacteria including *Haemophilus influenzae* and *Moraxella catarrhalis*. Whether a bacterial infection is a cause or consequence of disease exacerbation remains unclear. Recent studies show that the abundance of specific bacterial genera in the lung microbiome shifts as COPD severity increases - as COPD symptoms worsen, the abundance of commensal *Prevotella spp.* decreases, while the abundance of opportunistic pathogen *Moraxella spp.* increases. These shifts in the diversity of the microbiome are associated with lower expression of genes promoting epithelial barrier integrity, including those which promote tight junction formation. Tight junctions are important structures for maintaining epithelial integrity and are an important marker of lung health. The association between *Prevotella spp.* and *Moraxella spp.* abundance, and the expression of tight junction proteins, suggests that the composition of the microbiome might be able to directly affect epithelial integrity and therefore a direct link between the microbiome and disease outcomes.

The relationship between respiratory microbiome commensals and pathogens on the integrity of the respiratory epithelium was explored using Calu-3 monolayers grown at the air-liquid interface. Our data show that *P. melaninogenica* has a weaker potential to damage epithelial integrity than *M. catarrhalis*, and that this difference was visible in the intensity of occludin in Calu-3 monolayers when cells were fluorescently labelled. The impact of microbial diversity on important cellular processes in COPD will be discussed.

BLOCK A

Session : Single Cell Omics

A296

Characterizing LPS dynamics on Pseudomonas aeruginosa at the single cell level

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Abstract

Pseudomonas aeruginosa is a Gram-negative bacterium and an opportunistic human pathogen, posing a wide range of life-threatening infections on immunodeficient patients. The increasing challenge in treating the infections of *P. aeruginosa* can be attributed to its identified capacity to resist multiple antibiotics. Colistin, a polymyxin antibiotic serves as the last-resort therapeutic antibiotics against *P. aeruginosa* infections, targets lipopolysaccharides (LPS) on the outer leaflet of bacterial outer membrane. The negatively charged lipid A component of LPS interacts with the positively charged colistin, resulting in the destruction of membrane permeability. The acting efficacy of colistin can be sabotaged by LPS molecular modification. A comprehensive understanding of LPS on bacterial surfaces can provide insight to develop new antimicrobial strategies against infections. Stochastic optical reconstruction microscopy (STORM) is a super-resolution microscopy, breaking the resolution limitation of optical microscopy by stochastic localising and mathematical reconstructing individual fluorophores. The aim was to investigate LPS dynamics on *P. aeruginosa* at the single cell level using STORM. *P. aeruginosa* was subjected to stain with fluorophore-conjugated polymyxin in compared to stain with a lipophilic fluorescent dye. Results by from STORM and statistical analysis, indicated that the intensity of lipophilic stained cellular membrane was uniformly distributed, suggesting that it is an integral component of the entire cell membrane. LPS strained by fluorescent-polymyxin exhibited an intermittent distribution pattern, which implies an unequal distribution of LPS across the cell membrane. The current observation lay the groundwork for future investigations in the LPS distribution in potential contribution to colistin resistance.

BLOCK A

Session : Understanding phenotypes in the omics era

A297

New insights into Trichomonas - Bacteria Interactions through comparative Genomics, Transcriptomics and Biochemistry

Adam Hart, Jacob Biboy, Joseph Gray, Waldemar Vollmer, Robert Hirt

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Abstract

The Trichomonas genus represents a diverse group of parasitic protozoans which can infect a range of animal species with a well-established zoonotic potential. Species include Trichomonas vaginalis, a Human STI, and Trichomonas gallinae, which infects birds, primarily Columbiformes.

They reside at mucosal surfaces of their host, alongside a complex microbiota. Trichomonas species are described as able to damage host tissue through inducing excessive inflammatory host responses. Notably, infections of Human and birds by Trichomonas species are associated with significant changes in the microbiota taxonomic composition. In Humans, change associated with T. vaginalis infection of the female urogenital tract are considered to lead to a dysbiotic microbiota that contributes synergistically to disease states and increases susceptibility to important pathogens, namely HIV and HPV.

However, interactions between Trichomonas and the members of the microbiota are poorly understood at the molecular and cellular level. This work aims to gain new insights into Trichomonas-Bacterial interactions through integrating microbiological, biochemistry/enzymology, comparative genomic and transcriptomic approaches.

Using Trichomonas gallinae as a model we present evidence that Trichomonas species, including T. vaginalis, have acquired a repertoire of genes encoding enzymes capable of interacting with the bacterial cell wall that have their transcripts significantly modulated in the presence of the bacteria Escherichia coli.

These tools may allow Trichomonas to out-compete neighbouring bacteria and/or liberate molecules that can promote Trichomonas' growth and host tissue inflammations. These findings bring new insights into Trichomonas-Bacterial interactions and how these evolutionarily conserved interactions can potentially influence the zoonotic ability of Trichomonas.

A298

Using barcode sequencing to understand the genome dynamics of *Streptomyces clavuligerus* during industrial fermentations

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Abstract

Antimicrobial resistance has driven the requirement for the development of new antibiotics and a comprehensive understanding of the production of antimicrobials and the microorganisms that produce them. Clavulanic acid (CA) is a compound administered alongside beta-lactam antibiotics to increase their efficacy and is synthesised industrially by *Streptomyces clavuligerus*. Decades of strain improvement through random mutagenesis have allowed the development of strains of *S. clavuligerus* that return higher yields of CA, often at the cost of genetic stability and metabolic flexibility. Extensive genome sequencing efforts have shown that these strains have a dynamic genome that may be unpredictable during the fermentation process, significantly affecting the production of CA throughout the process. We have repurposed barcode sequencing technology which allows us to monitor the genotype of *S. clavuligerus* and relate to phenotypic changes within an industrial fermentation. This enables the tracking of emerging clonal lineages in the fermentation via the relative abundance of barcoded strains. We have shown that media and scale of a fermentation effects the stability of the strains, potentially altering their production of CA. This work was carried out with a view to using genetic interventions to improve the stability of strains within a fermentation, thus returning higher and more consistent industrial yields of CA to help us tackle the ongoing problem of antimicrobial resistance.

A299

Transcriptionally active nasopharyngeal commensals and opportunistic microbial dynamics define mild symptoms in the COVID-19 vaccination breakthroughs

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Abstract

The development of COVID-19 vaccines as an effort to mitigate the outbreak, has saved millions of lives globally. However, vaccination breakthroughs have challenged the vaccines' effectiveness and provided incentives to explore facets holding potential to alter vaccination-induced immunity and protection from subsequent infection. We explored functional dynamics of nasopharyngeal transcriptionally active microbes (TAMs) between vaccination breakthroughs and unvaccinated SARS-CoV-2 infected individuals. Microbial taxonomic communities were differentially altered with skewed enrichment of bacterial genera of *Firmicutes* and *Gammaproteobacteria* with grossly reduced phylum *Bacteroidetes* in vaccination breakthrough individuals. The *Bacillus* genus was abundant in *Firmicutes* in vaccination breakthrough whereas *Prevotella* among *Bacteroides* dominated the unvaccinated. Also, *Pseudomonas* and *Salmonella* of *Gammaproteobacteria* were overrepresented in vaccination breakthrough, whilst unvaccinated showed presence of several genera, *Achromobacter*, *Bordetella*, *Salmonella* and *Pseudomonas*, belonging to Proteobacteria. At species level, the microbiota of vaccination breakthrough exhibited higher abundance of unique commensals, in comparison to potential opportunistic microbes enrichment in unvaccinated patients' microbiota. Functional metabolic pathways like amino acid biosynthesis, fatty acid and beta oxidation, associated with generation of SCFAs (short chain fatty acids), were enriched in vaccination breakthroughs. Majorly, metabolic pathways of LCFAs biosynthesis (long chain fatty acids) were associated with the unvaccinated. Our research highlights that vaccination decreases the microbial diversity in terms of depleting opportunistic pathogens and increasing the preponderance of commensals with respect to unvaccinated patients. Metabolic pathway analysis substantiates the shift in diversity to functionally modulate immune response generation, which may be related to mild clinical manifestations and faster recovery times during vaccination breakthroughs.

A300

Intergenic regions with phenotypic consequences: Multiomics analysis of *Mycobacteroides abscessus*

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Abstract

Mycobacteroides abscessus (MABC) is capable of causing pulmonary, skin, soft tissue, and disseminated infections with immunocompromised patients (such as Cystic Fibrosis patients) being most at risk. Increasing numbers of these infections are resistant to treatment with antibiotics with resistance often being acquired from other microorganisms.

Early work by Sharples and Lloyd identified the potential importance of Intergenic Regions (IGRs) within bacterial chromosomes in relation to their flanking genes where intergenic repeat units are found in identical locations across *Escherichia coli* and *Salmonella typhimurium* suggesting potential conservation across the Enterobacteriaceae family. Despite this work, little advancement occurred in understanding IGRs in microorganisms until 2018 when a pangenome analysis tool for IGRs in bacteria was published called Piggy. Piggy is solely for IGRs and allows for examination of horizontal gene transfer as well as expanding our ability to infer the impact of gene regulation on phenotypic expression.

Presence/absence of clusters of homologous intergenic sequences in GWASs enable a better understanding of genetic influences on expressed phenotypes and can be utilised to explore regions of a species' pangenome to identify antimicrobial resistance genes that could have been transferred by mobile genetic elements.

This study identified significant genes related to antibiotic resistance, including TetR/AcrR regulators, beta-lactamases, MarR family, WhiB, and PaaX. Phage-driven antimicrobial resistance evolution was highlighted, with both lytic and lysogenic phage playing roles, particularly lysogenic phage carrying AMR genes. The presence of genes foreign to MABC, such as SpoIIM, ComA, and FlgA, raise questions about their origins and potential functions.

A301

Molecular mechanisms underpinning the adaptation to defects in cell cycle control regulators in *Schizosaccharomyces pombe*

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Abstract

The eukaryotic cell cycle is a controlled and regulated process, enabling cells to grow and divide into two identical daughter cells. Cdc25 phosphatase and Wee1 kinase regulate the entry of cells into the mitotic phase of the cell cycle by engaging in a bistable molecular switch to abruptly activate the cyclin-dependent kinase, Cdc2. The cell cycle has the capacity to evolve and adapt as seen in cancers; as such understanding mechanisms of cell cycle control adaptation could be pertinent to uncover potential treatment targets or resistance pathways. In this study, using experimental evolution and the fission yeast Schizosaccharomyces pombe as a model organism for cell cycle control, we determine how cell cycle regulation can adapt when defective. Wild type (PN1) and mutant (wee1Δ and cdc25-ts (temperature sensitive)) strains of the fission yeast were evolved for over 200 generations and were phenotypically characterised (cell size at division, generation time, and temperature sensitivity). Minimal changes were seen in the evolved wild type and wee1Δ populations, but significant phenotypic differences were observed in the evolved cdc25-ts populations compared to their ancestors, with cell size at division, generation time and temperature sensitivity all decreasing. Notably, there were variations in phenotypes in the evolved cdc25-ts single clones, suggesting that there are multiple adaptation pathways to defects in mitotic entry and cell cycle control. Initial genotypic analysis of the cdc25-ts populations suggests evolution is constrained, with one population containing a mutation in cdc2 and two other populations with mutations in the protein kinase domain of wee1.

A302

Gut microbiome-metabolome interactions predict host condition

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Abstract

Background The effect of microbes on their human host is often mediated through changes in metabolite concentrations. As such, multiple tools have been proposed to predict metabolite concentrations from microbial taxa frequencies. Such tools typically fail to capture the dependence of the microbiome-metabolite relation on the environment.

Results. We propose to treat the microbiome-metabolome relation as the equilibrium of a complex interaction and to relate the host condition to a latent representation of the interaction between the log concentration of the metabolome and the log frequencies of the microbiome.

We develop LOCATE (Latent variables Of miCrobiome And meTabolites rElations), a machine learning tool to predict the metabolite concentration from the microbiome composition and produce a latent representation of the interaction. This representation is then used to predict the host condition.

LOCATE's accuracy in predicting the metabolome is higher than all current predictors. The metabolite concentration prediction accuracy significantly decreases cross datasets, and cross conditions, especially in 16S data. LOCATE’s latent representation predicts the host condition better than either the microbiome or the metabolome. This representation is strongly correlated with host demographics. A significant improvement in accuracy (0.793 vs. 0.724 average accuracy) is obtained even with a small number of metabolite samples (~ 50).

Conclusions. While microbiome data is often very high dimensional, and as such suffers from a multiple measurement problem when compared with a phenotype, the taxonomic structure can be used to mitigate this limitation and ensure a high discovery rate with a very low false discovery rate.

A303

Deciphering the Mechanisms of Faecal Microbiota Transplantation Success: A Groundbreaking Clonal-Level Engraftment Pipeline

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Abstract

Faecal Microbiota Transplantation (FMT) has emerged as a groundbreaking therapeutic approach for various medical conditions, utilising the transfer of gut microbiota to restore microbial balance. In this study, we employ a novel clonal-level engraftment pipeline to comprehensively analyse multiple public FMT studies across diverse medical conditions. Our primary focus centres on investigating the association between engraftment and clinical phenotypes, including treatment outcomes and patient characteristics.

Through our analysis, we have identified specific bacterial species exhibiting high engraftment effectiveness, providing critical insights into the dynamics of FMT. Additionally, we have elucidated engraftment patterns by performing a comprehensive examination of phages, functional potential, and within-species variations. Finally, we investigated whether engraftment success is influenced more significantly by the recipient, the donor, or a combination of both. This holistic approach has enabled us to uncover key factors influencing the success of bacterial engraftment and treatment, thus paving the way for a deeper understanding of FMT mechanisms.

Our research marks a significant advancement in FMT methodologies, offering innovative perspectives on microbial transfer and its clinical ramifications. By revealing the intricate interplay between engraftment efficiency and clinical outcomes, this research holds the potential to optimise the development of FMTs and other live biotherapeutic treatments, ultimately enhancing the efficacy of microbiota-based therapies across diverse medical contexts.

A305

A high-resolution genomic and phenotypic analysis of resistance evolution of an Escherichia coli strain from a critical care patient treated with piperacillin/tazobactam .

Alice Fraser[1], Robert Ball[2], Daire Cantillon[1], Laura Brettel[1,3], Fabrice Graf[1], Joseph Lewis[1,2,4], Jon van Aartsen[2,5], Christopher Parry[1,6], Eva Heinz[1], Thomas Edwards[1]

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Abstract

Resistance to the β-lactam/β-lactamase inhibitor (BL/BLI) combination antibiotic piperacillin/tazobactam (TZP) predominantly occurs with resistance to third-generation cephalosporins (3GCs). However, if β-lactamases which would otherwise not confer TZP or 3GC resistance are expressed at high levels that result in enzyme hyperproduction, the surplus enzyme can selectively escape tazobactam inhibition. Furthermore, the mechanism by which hyperproduction occurs can be inducible upon antibiotic administration, resulting in treatment failure despite data from initial antimicrobial susceptibility testing supporting TZP use.

We identified an *E. coli* isolate, which evolved resistance to TZP during patient treatment. Our whole genome sequencing analyses show that resistance to TZP evolved via hyperproduction of TEM-1 β-lactamase, induced by extensive IS*26*-mediated duplication of a $bla_{TEM\text{-}1}$ containing gene cassette, located on a plasmid. We demonstrate that ten copies of $bla_{TEM\text{-}1}$ induce resistance greater than 32-times the MIC and exposure of the resistant isolate to TZP further increases amplification of $bla_{TEM\text{-}1}$. Furthermore, in the absence of TZP, gene copy number of *IS26* and $bla_{TEM\text{-}1}$ remains stable over five days, despite a 48,205 bp increase compared to the pre-treatment isolate. We additionally detect phenotypic changes that might indicate host adaptation and identify potential causes due to the duplication of regulatory genes within the gene cassette.

Infections caused by isolates which evolve to hyperproduce β-lactamases represent a complex problem, specifically regarding their detection and treatment. Our analysis advances the understanding of this resistance-mechanism, however, further investigations are of urgent concern as 40% of antibiotics active against WHO priority pathogens in the pre-clinical pipeline are BL/BLI combinations.

A306

Molecular mechanisms of re-emerging chloramphenicol susceptibility in extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract

Infections with extended-spectrum beta-lactamase (ESBL) producing Enterobacterales (E) are challenging to treat, especially in low-income settings. After ceftriaxone, a 3rd-generation cephalosporin, replaced chloramphenicol (CHL) as empiric therapy for suspected sepsis in Malawi in 2004, ESBL-E rapidly emerged. In contrast, resistance to CHL in *Escherichia coli* and *Klebsiella* spp. decreased as its use fell, raising the possibility of its re-introduction. However, many phenotypically susceptible isolates had a genotype indicative of resistance as they carry predicted CHL acetyltransferases (cat) genes.

We used a combination of functional assays, experimental evolution, and genomics, to explore those genotype-phenotype mismatches.

We assembled a collection of 840 Malawian isolates of which 31% had discordant CHL susceptibility genotype-phenotype and selected a subset of 42 isolates for in-depth analysis. We found that the dominant *catB3* gene has been truncated by insertion sequence IS*26*, this had been previously annotated as *catB4* but is non-functional. Further, integration of an IS*5* element into the promoter of *catA1* interrupted its transcription. Both insertions are stable and CHL resistance does not rapidly emerge when exposed to increasing concentration of CHL. The truncated *catB3* is globally widespread and investigating the context of sequence types indicated an association with dominant lineages of *E. coli* possibly explaining the high proportion of CHL susceptibility in ESBL-E populations in Malawi.

Our study suggests CHL could be re-introduced as a reserve agent for critically ill patients with ESBL-E infections in Malawi and similar settings and highlights the ongoing challenges in inferring antimicrobial resistance from sequence data.

A307

Structural Characterisation of Lysogenic Phages from the Liverpool Epidemic Strain of *Pseudomonas aeruginosa* and their Influence on Type Six Secretion Systems

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Abstract

The Liverpool Epidemic Strain (LES) of *Pseudomonas aeruginosa* is a key opportunistic pathogen and a major cause of respiratory morbidity and mortality in cystic fibrosis (CF) patients. A set of active prophages has been associated with the fitness advantages of LES. Transcriptomic studies revealed that each LES phage affects the expression of *Pseudomonas* host genes differently during polylysogeny of the well-characterised PAO1 strain. A range of virulence-associated genes were affected, including several genes belonging to Type Six Secretion Systems (T6SS).

This study carried out structural analysis of LES phages 2, 3 and 4 by transmission electron microscopy (TEM), confirming Siphoviridae morphology with icosahedral capsid heads (50-60 nm diameter) and long flexible tails (~200 nm long). Tail fibre structures were clearly visible at the end of LES phage 4 tails. Proteomic analysis of purified LES phage suspensions by SDS page detected 26, 13 and 13 structural proteins for LES phages 2, 3 and 4, respectively, which is more than could be identified using genome annotation tools. Functional competition assays can be used to investigate whether prophage-associated changes in T6SS genes affected PAO1 killing of *Escherichia coli* in co-culture. Preliminary results suggest that carriage of LES phages 3 or 4 individually or in combination affects the rate of killing of *E. coli* in co-culture. However, overall numbers were too low for robust statistical analysis, and further method optimisation is required.

Overall, this study has used a combination of genomic, proteomic, TEM and functional analyses to improve our understanding of LES phage biology.

A308

Convergence of resistance and evolutionary responses in Escherichia coli and Salmonella enterica co-inhabiting the same host

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Abstract

Sharing of genetic elements among different pathogens and commensals inhabiting the same hosts and environments has significant implications for antimicrobial resistance (AMR), especially in settings with high antimicrobial exposure. We analysed 661 *Escherichia coli* and *Salmonella enterica* isolates collected within and across hosts and environments, in 10 Chinese chicken farms over 2.5 years using novel data-mining methods. Most isolates within the same hosts possessed the same clinically relevant AMR-carrying mobile genetic elements (plasmids: 70.6%, transposons: 78%), which also showed recent common evolution. Machine learning revealed known and novel AMR-associated mutations and genes underlying resistance to 28 antimicrobials and primarily associated with resistance in *E. coli* and susceptibility in *S. enterica*. Many were essential and affected the same metabolic processes in both species, albeit with varying degrees of phylogenetic penetration. Multi-modal strategies are crucial to investigate the interplay of mobilome, resistance and metabolism in cohabiting bacteria, especially in ecological settings where community-driven resistance selection occurs.

A309

Do plant polysaccharides influence microbiome assemblage?

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Abstract

Through the release of exudates, plants manipulate the soil surrounding their roots, creating a region called the rhizosphere. Microbiota inhabiting the rhizosphere are typically well adapted to utilise these root exudates, which are composed of low molecular weight and high molecular weight (HMW) molecules. Whilst there have been significant advances toward understanding the composition of the plant rhizosphere microbiome, the importance of HMW polymers, e.g., polysaccharides, in driving assemblage is very limited. *Bacteroidota* represent an abundant bacterial phylum found in the soil and are typically enriched in the plant microbiome. In the human gut, Bacteroidota are key polysaccharide degraders, including various dietary plant polysaccharides. However, the role of soil and plant-dwelling Bacteroidota on plant polysaccharides has received less attention. Bacteroidota possess specialised gene clusters, termed polysaccharide utilisation loci (PUL). These PULs encode proteins with functions relating to the utilisation of complex carbohydrates. However, the exact mechanisms enabling *Bacteroidota* to degrade complex carbohydrates in the soil and succeed in this niche are unknown.

My work has identified specific PULs associated with plant polysaccharide utilisation and investigated the role this unique metabolism has on plant microbiome assemblage. Using *Flavobacterium* spp. as the model and combining bacterial genetics, proteomics and light microscopy, I have shown plant polysaccharide utilisation provides *Flavobacterium* with a competitive advantage when inhabiting the plant microbiome. Given *Flavobacterium*, as well as other Bacteroidota have an important role in plant disease suppression, I plan to further investigate any direct or indirect interactions occurring between *Flavobacterium* and bacterial root pathogens, such as *Xanthomonas* and *Ralsontia.*

A310

Volatile metabolite profiles from the culture headspace of azole-resistant *Aspergillus fumigatus*

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Abstract

Accurate and rapid identification of azole-resistance in *Aspergillus fumigatus*, the most commonly isolated pathogenic species in Pulmonary Aspergillosis, is critical for early initiation of effective treatment, prevention of dissemination and patient outcomes. Current culture-based methods for identification and susceptibility testing are slow, PCR is rapid but does not detect resistance, and biomarkers such as galactomannan lack specificity.

Aspergillus fumigatus produces several chemically diverse microbial volatile organic compounds intrinsic to cellular metabolic pathways. Some *Aspergillus* VOCs have demonstrated ability to discriminate colonisation from patients with invasive Aspergillosis. This study explored the VOC profile using Gas Chromatography-Mass Spectrometry of *A. fumigatus* cultures with variable azole-resistance patterns. We show that VOCs of azole-resistant isolates differ from azole-sensitive ones.

A311

Deciphering COVID-19 Molecular Pathways: Using The TopMD Algorithm To Identify Biomarkers In Patient Cohorts

Rebekah Penrice-Randal ORCID iD[1,2], Fabio Strazzeri[2], Catherine Hartley[1], James Schofield[2], Paul Skipp[3], Diana Baralle[3], Julian Hiscox[1,4]

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Abstract

The COVID-19 pandemic has placed a strain on healthcare systems worldwide. Tools that can stratify individuals according to prognosis or molecular phenotypes could allow for more efficient allocation of healthcare resources and thus improved patient outcomes. Our previous studies, using topological analysis (TopMD), have shown that gene expression data derived from whole blood at the time of admission to hospital can be used to predict admission to the intensive care unit (ICU). Rather than using a traditional binary approach such as DGE, our TopMD platform utilises every measured data point to interrogate biological pathway activation. Whole blood was collected in PAXgene tubes from patients hospitalised with COVID-19 in Liege and Florence at point of admission and 48 hours alongside clinical and CT scan data within the DRAGON consortium. RNA was extracted from blood prior to globin and rRNA depletion then RNA sequencing libraries were sequenced on the NovaSeq 6000 (Illumina). Paired-end fastq files were trimmed with fastp, and transcripts were quantified using Salmon where gene expression was inferred using the R package tximport. The host response within our cohorts was then assessed with traditional transcriptomic approaches and TopMD. TopMD molecular phenotype maps were generated for individual patients and sub-phenotypes of COVID-19 disease was explored. Finally, we conducted a multifactorial analysis including gene expression, pathway activation derived from TopMD analysis, CT scan data, and clinical measurements.

A313

Unveiling the Interplay Between Alzheimer's Disease Subphenotypes and the Gut Microbiome: A TopMD Approach

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Abstract

Personalised medicine necessitates a comprehensive understanding of the molecular underpinnings of diseases. Traditionally, disease classification has relied on single gene biomarkers, but these approaches often fail to capture the complex interplay of multiple pathways. TopMD, an AI-enhanced technology, analyses the topology of global gene expression to identify activated pathways, providing a more accurate representation of the molecular phenotype.

In this study, we employed TopMD to investigate the relationship between enriched biological pathways in Alzheimer's disease (AD) patients and their gut microbiomes. AD patients and matched controls were analyzed using long-read sequencing (Oxford Nanopore Technologies) to assess their blood transcriptomes. Our approach identified three distinct sub-phenotypes of AD based on their transcriptomes. Further exploration of the gut microbiomes revealed specific differences between these sub-phenotypes, suggesting a potential link between AD subphenotypes and gut microbiota composition.

This study highlights the utility of TopMD and multi-omics data in unraveling the intricate relationship between disease subphenotypes and the gut microbiome. Our findings pave the way for personalised medicine approaches that account for the unique molecular signatures of individual patients.

A314

Genome wide association reveals bacterial factors linked to cholera severity in Bangladesh between 2014 and 2018

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Abstract

The severity of cholera disease is believed to arise from the interplay between environment, host and pathogen genetics. However, the clonality of outbreaks presents a major challenge in identifying pathogen factors associated with severe disease, which is characterised by rice-watery stool and severe dehydration. Here we carry out a bacterial genome wide association study to identify *Vibrio cholerae* variants associated with severe dehydration and rice watery stool in Bangladesh from 2014-2018. The presence/absence of sequences in *V. cholerae* genome assemblies were determined using unitig-caller, and a generalised linear model was run for each unitig using pyseer v1.3.3. After adjusting for population structure, site of collection, patient age and sex and multiple testing, 87 unitigs were significantly associated with severe dehydration (Bonferroni adjusted $p < 0.05$). Analyses showed that 52 of these unitigs mapped to the VSP-II pathogenicity island, of which 30 were intragenic. Three mapped to the *rfbT* gene that determines serotype. 12 unitigs were associated with rice watery stool, including one mapping to cytotoxin *rtxA*. Collectively our results suggest that *V. cholerae* O1 genetics play a substantial role in the clinical presentation of cholera.

A315

A unique gene cluster enables phospholipid utilisation in a forest soil bacterium

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Abstract

In terrestrial and aquatic ecosystems, phosphorus (P) availability controls primary production, with consequences for climate regulation and global food security. Understanding the microbial controls on the global P cycle is a prerequisite for minimising our reliance on non-renewable phosphate rock reserves and reducing pollution associated with excessive P fertiliser use. Of particular importance is the role microbes play in remineralising plant-available phosphate from immobilised organic P complexes. The phylum Bacteroidota play important roles in complex carbon cycling, suppressing plant diseases, and transforming organic P into labile phosphate. This involves synthesising a unique phosphatase enzyme (PafA), which outperforms classical phosphatases in performing this desirable P-liberating process.

Initial protein biochemistry data revealed there are significant differences in enzyme kinetics between PafA forms from different representatives of the Bacteroidota phylum, with AlphaFold modelling suggesting gross changes in active site structure, despite sharing conserved catalytic residues. One distinct form encoded in the forest soil bacterium *Chitinophaga pinensis*, is found within a unique gene cluster enabling this bacterium to utilise phospholipid headgroups that other Bacteroidota, such as *Flavobacterium*, cannot. *Chitinophaga* spp. are abundant in natural soil ecosystems, and we propose they are key players in phospholipid recycling and increasing soil P availability. Proteomic analysis of the growth of *Chitinophaga* spp. on a variety of phospholipid substrates has enabled some initial characterisation of the phosphorus utilisation capabilities of these key microbes that could progress innovative approaches for enhancing sustainable agriculture.

A316

A Co-transcriptional Regulatory Mechanism Tightly Controls Gene Expression during Stress in Budding Yeast

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Abstract

Nab3, Nrd1 and Sen1 are RNA-binding proteins that form the Nab3-Nrd1-Sen1 (NNS) transcriptional termination complex in *Saccharomyces cerevisiae*. Intriguingly, during adaptation, some mRNAs are simultaneously targeted for transcriptional initiation and premature termination by the NNS complex. We hypothesised that it must be beneficial for the cell to keep low and tightly regulated expression of these genes during nutrient deprivation. To test this, we selected a stress-specific target of the NNS complex, *PIC2*, which encodes a mitochondrial phosphate and copper importer. After disrupting the RNA-binding sites of Nab3 in *PIC2*, we applied microfluidics and time-lapse microscopy to show that, indeed, the levels and variability in the expression of the gene increased. Importantly, these changes were accompanied by growth defects, enhanced cell size, mitochondrial hyperpolarisation, and resistance to oxidative stress.

To determine whether these phenotypes solely emerged from an increase in the activity of Pic2, we generated and characterised a *PIC2* overexpression mutant, which only recapitulated the mitochondrial malfunctions and, strikingly, displayed hypersensitivity to environmental challenges. Although this evidence proved that maintaining an optimal expression of *PIC2* is critical to increase microbial fitness during stress, it also showed that larger levels of Pic2 did not underlie all the observed defects. Transcriptomic, proteomic and metabolomic profiling of the mutant lacking Nab3 RNA-binding sites in *PIC2* uncovered a cell-wide accumulation of NNS targets. Our findings illustrate that a modest excess of one RNA target is sufficient to disturb the homeostasis of co-regulated transcripts and so decrease fitness.

A317

Mechanisms of antimicrobial resistance in *Helicobacter pylori*

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Abstract

The difficult treatment and progression of stomach cancer is attributed to the widespread presence of *Helicobacter pylori* infection, coupled with growing levels of antimicrobial resistance (AMR). This project aimed to characterise within-population variations in AMR genotypes and phenotypes, using multiple *H. pylori* single colony isolates from individual patient's stomachs, and to investigate associations between AMR and wider genomic factors.

Twenty-two single colony isolates were studied, obtained from gastric biopsy samples of both the antrum and corpus regions of the stomach, from two different patients attending the Queen's Medical Centre, Nottingham.E-test strips were used to determine the susceptibility of the isolates to clarithromycin, metronidazole, tetracycline, levofloxacin, amoxicillin, and rifampicin. Using the bioinformatics tools, Bakta and RGI, the corresponding assembled genomes were annotated, and the antimicrobial resistance genes identified. Publicly available datasets of susceptibility data and assembled genomes of *H. pylori* strains were retrieved from the literature. This combined with our own data, was used to investigate the associations of susceptibility profiles with wider genomic factors.

We found the presence of some variations in antibiotic resistance phenotypes and different antibiotic resistance genotypes among the *H. pylori* populations within individual patient's stomachs, which might contribute to eradication therapy failure. Discordance between the genetic and phenotypic antibiotic resistance profiles of the isolates was also detected in some cases. Phylogenetic analysis of AMR genes revealed that most *H. pylori* single colony isolate genomes grouped by their patient of origin, rather than stomach location.

A318

Surveillance and phenotypic characterization of antimicrobial resistant bacteria in milk and dairy environments

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Abstract

It is estimated that AMR bacteria caused 1.27 million deaths in 2019, and this number is expected to increase to 10 million in every year. Livestock and farming practices have been implicated as contributors to the AMR problem due to misuse or overuse of antibiotics. Moreover, bovine milk can act as reservoirs of microbial growth and their associated antimicrobial resistant pathogens posing a significant public health threat. A diverse microbial community with over 150 different bacterial strains is thought to reside in milk and genes including AMR genes (ARGs) from these organisms can be transmitted to humans by cross contamination or direct consumption of milk and dairy products.

In our study, we aim to explore the natural microbial populations in milk using multi omic and culturomics approaches. By studying the structural and functional diversities of milk microbial community and identifying transmissible ARGs, we can provide insight into the risk of AMR transmission in dairy production. We will also perform meta-analyses of the microbial ecology of milk from commercial farms in Northern Ireland and Scotland as influenced by environmental factors and profile the transcriptome of zoonotic and indicator organisms in milk populations.

This identification of complex microbial communities advances our understanding of the impact of production environment on the structure, composition, and phenotypes of natural microbial populations of milk and will also elucidate the zoonotic and indicator organisms associated with milk and their antimicrobial resistance phenotypes. This is ultimately crucial for development of intervention strategies to minimize the entry of such AMR pathogens and genes into the food chain thereby lessening the health hazards to achieve sustainable development goals 2 and 3.

A319

Systemic proteomic analysis of human IFNλ stimulation reveals novel antiviral mechanisms.

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Abstract

Type III interferons, or interferon lambdas (IFNλ), are a first-line of defence against viral infections of epithelia. IFNλs act via a distinct receptor but have hitherto been thought to functionally resemble type I interferons. Both activate intracellular signalling pathways and antiviral functions, and both are induced by viral infection. IFNλ4 expression is associated with reduced disease clearance of a number of biologically relevant viral infections, including hepatitis C and influenza A viruses.

We utilised primary-like human bronchial KT cells, which retain the ability to differentiate into ciliated epithelia, as a tractable, physiologically relevant model for dissecting the response to type I IFN as well as IFNλ1-4 and human hypo- and hyper-functional λ variants. Systemic highly multiplexed tandem-mass-tag-based proteomics was used to quantify ~8,000 proteins precisely over time. STAT1, IFITs, and multiple other known or unknown interferon-stimulated proteins were induced by all IFNs in a qualitatively similar but quantitatively different fashion. Fascinatingly, we also observed λ-variant specific responses which may correlate with clinical outcomes. As viruses often regulate key cellular antiviral proteins, we compared our data to a compendium of our proteomic studies across multiple different viral infections to discover factors that are both stimulated by IFNλs and regulated by viral infection.

Overall, we have identified novel proteins induced in response to specific IFNλs, which may inform basic IFN biology, as well as a subset of IFNλ-stimulated proteins regulated by DNA and/or RNA viruses which may represent critical novel innate immune factors.

A320

From Infection to Protection: How Prophages Contribute to Antiviral Defence in *Pseudomonas aeruginosa*

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Abstract

Viruses that infect bacteria (phages) are the most abundant biological entities on this planet and play key roles in microbial evolution and the composition and function of microbial communities. In recent years, it was discovered that bacteria dedicate a significant portion of their genome to anti-phage defences. Anecdotal evidence suggests that defence genes can also be located on prophages (phages that integrate into the bacterial genome), but systematic studies that examine their contribution to bacterial immunity are currently lacking. This project aims to unveil the role of prophage-encoded defence systems in shaping bacterial resistance against lytic phage infections. Using *Pseudomonas aeruginosa* as a model organism, we isolated temperate phages from a unique collection of 300 clinical isolates. Then we generated lysogens in a “defenceless” mutant of the PAO1 lab strain, where prophages and all known defence systems were removed. Currently we are challenging lysogen collection by 30 different lytic phages to identify defensive phenotypes. We are integrating experimental data with bioinformatic analysis of prophage genomes. This approach aims to identify the genetic basis of a prophage-caused anti-phage resistance.

A321

Combining experimental evolution and whole genome sequencing to study antimicrobial resistance gene (ARG) transfer in clinical Methicillin-Resistant Staphylococcus aureus (MRSA) isolates.

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive commensal of the nose, and opportunistic pathogen. In recent global estimates, MRSA was responsible for over 100,000 deaths attributable to antibiotic resistance in 2019.

MRSA populations can rapidly adapt to new niches and selective pressures such as antimicrobial exposure via generalised transduction by endogenous bacteriophage. Occasional packaging of host bacterial DNA into phage particles results in delivery and integration of donor DNA into a recipient.

We aim to establish a new laboratory model of AMR gene transfer between clinical MRSA isolates to uncover patterns of gene transfer between circulating strains. Bioinformatic analyses of MRSA whole genome sequences will uncover genetic components responsible for horizontal gene transfer (HGT) in clinical MRSA samples. Combining AMR gene transfer phenotype with genomic data will uncover mechanisms behind efficient and stable AMR gene transfer in MRSA.

Results

Whole genome sequencing data of a European clinical MRSA isolate collection is assembled and screened for AMR genes, endogenous bacteriophage, and HGT-related genes.

In a competitive gene transfer assay, some isolates transfer AMR genes with all tested partners, some only with a subset of partners, and some exhibit no transfer phenotypes with all partners. Candidate genes identified by genome screening are associated with experimental results to explain phenotypic variation.

Significance

Enhancing our understanding of phage-driven AMR gene transfer mechanisms in clinical MRSA populations explains evolutionary and epidemiological dynamics. Results may have implications in clinical diagnostics and development of alternative therapies (including phage therapy).

BLOCK A

Session : Therapeutics: the use of bacteriophage, viruses, and viral components

A325

Characterising novel bacteriophage receptors of invasive non-typhoidal Salmonella.

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Abstract

Invasive non-typhoidal Salmonella (iNTS) has emerged as a major cause of bloodstream infection in sub-Saharan Africa, posing a significant threat to public health. The rise of antimicrobial drug resistance intensifies the challenges of treating iNTS infection, necessitating innovative therapeutic approaches. Bacteriophages (phages) hold promise as a potential solution; however, the broader acceptance of bacteriophages as therapeutic agents hinges on a deeper understanding of the bacteria-phage interactions. A substantial knowledge gap exists regarding bacteriophage interactions with iNTS. Therefore the aim of this project was to identify the specific surface receptors that phages use to infect *S.* Enteritidis, a prominent serovar responsible for one third of iNTS infections in Africa.

To identify the specific surface receptors that phages use to infect *S.* Enteritidis, multiple infection experiments were performed to isolate *S*. Enteritidis bacteria that had evolved to overcome phage predation. Resistance to phage was confirmed using phage-spot assays, and the genomic DNA of resistant strains was extracted for a genome-level analysis of the resistance mechanism.

Compared to other Salmonella isolates that have previously been studied in our group, it proved to be more challenging to isolate stable phage-resistant mutants in *S.* Enteritidis than anticipated. This raised the possibility that the mutations required to counter phage infection may be detrimental to the normal functioning of *S*. Enteritidis, providing a novel insight into the biology of this bacteria, and highlighting that potential trade-offs exist between phage resistance and normal bacterial function. Our findings emphasise the complexity and diversity of phage-host interactions in African Salmonella.

A326

In vitro activity new nitrofurans analogs against ESKAPE pathogens

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Abstract

Background. In recent years, particularly after the pandemic of COVID-19, antibiotic resistance has developed rapidly among the ESKAPE group. This leads to forced therapy of patients with maximum doses of antimicrobials, which entails a number of unfavorable consequences. In this regard, it's necessary to promptly search and study new antibacterial compounds, including modifications of existing antibiotics.

Objectives. The aim of our work was to study the antibacterial properties of 5-Nitrofuran-Tagged Oxazolyl Pyrazolopiperidines against the ESKAPE panel.

Methods. 8 synthetic substance were tested against multidrug-resistant bacteria of ESKAPE group isolated from ambulatory and in-patients in Saint Petersburg in primary screening by Kirby-Bauer method and then were determined minimal inhibitory concentrations meanings according to the EUCAST 13.0. Ciprofloxacin and nitrofurantoin were used in these assays as a positive control.

Results. The lead compounds among this set were substances LK0513, LK1509, LK1514, suppressing the growth of mainly 5 out of 6 pathogens. On the basis of the performed calculations, we can conclude that the compound LK01509 can be used — NfsB protein as the primary target and NfsA protein as the alternative target — due to the similarity in binding profiles with the control compounds. Activity loss is linked to the imbalanced interaction potential of the N-linked aliphatic substituent of the pyrazolopyridine scaffold. In the case of LK01509, LK01513, and LK01514, the sidechain per-atom binding potential is −5.83, −6.84, and −9.78 kcal/mol, given by lipophilic interactions. The growing role of lipophilic interactions with following amino acids leads to a binding pose rearrangement with target activity loss following.

A327

Quaternary ammonium N,N,N-trimethyl chitosan derivative and povidone-iodine complex as a potent antiseptic with enhanced wound healing property

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Abstract

The importance of developing more potent antimicrobials and robust infection prevention practices has been highlighted recently with the increase in reports of emerging bacterial resistance mechanisms and the development of antibiotic-resistant microbes. In this study, a quaternary ammonium chitosan derivative, N,N,N-trimethyl chitosan chloride (TMC) with inherent bactericidal property was synthesized and complexed with povidone-iodine (PVP-I) to create a potentially more potent antiseptic solution that could also significantly enhance the wound healing process. TMC, a positively charged, water-soluble derivative of chitosan, formed stable solutions with PVP-I at 5% w/v TMC concentration (TMC5/PVP-I). TMC5/PVP-I was significantly effective against multidrug-resistant bacteria S. aureus compared with PVP-I alone. TMC/PVP-I solutions also showed fungicidal property against C. albicans, with no cytotoxic effects when tested against human fibroblast cells cultured in vitro. Wound healing assessment in vivo revealed early collagen formation and re-epithelialization for TMC5/PVP-I treated wounds in rats relative to control and PVP-I only. Formulation of TMC/PVP-I solutions presented in the study can be easily adapted in the existing production of commercial PVP-I creating a new product with more potent bactericidal and enhanced wound healing properties for optimal wound care.

A328

NOVEL ANTI-MICROBIAL SMALL MOLECULE PHOTOSENSITISER TO TREAT RESISTANT GRAM-POSITIVE INFECTIONS

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Abstract

Antimicrobial Photodynamic Therapy (aPDT) uses light absorbing photosensitisers and light to illicit production of Reactive Oxygen Species (ROS); these toxic species indiscriminately react with components of the bacterial cell, circumventing typical resistance mechanisms and leading to loss of viability.

LightOx has developed a range of low molecular weight, fluorescent photosensitisers capable of passive uptake into bacterial cells and LightOx Compound A was used in this work. We tested Compound A for bacteriostatic and bactericidal action and impact on growth inhibition in a range of bacterial species and used confocal microscopy to determine compound uptake and localization in these species.

Compound A facilitates aPDT showing cell death (10^5 fold reduction in CFU/ml) in the MRSA strain USA300 at 2 µM. In *E. coli,* significantly less cell death is observed but a strong growth inhibition effect is seen. Fluorescence microscopy showed the localisation of the compound on cell membranes in gram-negative bacteria, whereas in gram-positive bacteria cytosolic fluorescence is seen. A lipopolysaccharide knockout (ΔwaaC) shows high susceptibility (10^6 fold reduction in CFU/ml) suggesting a role of the outer membrane in restricting Compound A entry. Knockouts of genes related to ROS detoxifying and DNA repair show more susceptibility and provide evidence for the ROS based mechanism of Compound A.

Here we show a novel small molecule which can penetrate and cause the death of Gram-positive bacteria after irradiation. Compound A has potential as a therapeutic treatment for drug resistant bacterial infections, such as chronic wounds.

A329

It's not all about the bacteria: Developing a cell-free microbiome-based therapy for the biocontrol of *Clostridioides difficile* in the gut.

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Abstract

Clostridiodes difficile is a nosocomial pathogen and is the leading cause of hospital acquired diarrhoea. An infection is primarily treated with broad spectrum antibiotics, however antibiotic-induced dysbiosis of the microbiome can lead to a high incidence of recurrent *C. difficile* infections (rCDI). Faecal microbiota transplants (FMT) have been used successfully to treat rCDI and other gastrointestinal diseases, but carry a risk of transferring unknown pathogens from the donor. There have been major developments into alternatives of FMT where the whole faecal sample is not used. Certain components of the microbiome such as specific bacteriophages or the virome in the faecal water have been used successfully to prevent rCDI.

This project aims to develop a cell-free-microbiome-based therapeutic to replace FMT. Cell-free faecal filtrates from healthy donors have been produced and used in *in vitro* colon models. The filtrates were incubated with different healthy donors' flora in the presence and absence of vegetative *C. difficile* cells. Initial results show that the filtrate does not have a direct effect on the growth of *C. difficile*. To fully understand the potential effect the filtrate has on the whole microbiome, whole-genome-shot-gun sequencing has been used to determine the composition (MetaPhlAn). Further approaches of metabolomic (^{1}H-NMR) and functional genomic analysis (Humann3) will determine changes to the functional profile of the microbiome. Viromic analysis of the filtrate (geNomad, CheckV, and vConTACT) will be used to investigate the diversity of the virome and whether this influences the way the microbiome is modulated.

A330

ACRIDONE-LIKE DRUGS BASED ON BRAZILIAN NATURAL SCAFFOLDS STRONGLY INHIBIT SARS-CoV-2 REPLICATION

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Abstract

Background: SARS-CoV-2 is the etiological agent of COVID-19, a disease that still burdens the health systems despite vaccination and the use of repurposed drugs. Therefore, it is essential to identify molecules that can inhibit SARS-CoV-2 replication. Here we evaluated the synthetic acridones FAC2, 4, 8, and 21, based on natural scaffolds from Brazilian plants, against SARS-CoV-2 infection. **Methods:** Dose-response and time-of-addition assays were performed with each compound in A549-AT cells infected with a SARS-CoV-2-Wuhan infectious clone expressing MCherry (SARS-CoV-2-MCherry). Broad-spectrum activity was evaluated using SARS-CoV-2-MCherry infectious clone chimeras expressing Delta or Omicron spikes, as well as both wild-type variants. Inhibition of cell-cell spread was also evaluated. Lastly, to determine the molecular targets, FACs-resistant viruses were selected by serial passage and analyzed by NGS. **Results:** FAC2, 4, 8, and 21 had selectivity indices of 42, 16.9, 5.4, and 16.9. All compounds resulted in over 90% reduction of post-entry steps, and inhibition was recovered when compounds were added after 6h of infection. FAC2, 4, and 21 decreased the titres of Delta and Omicron by one-log and 3-logs, respectively. FAC8 did not inhibit the Delta variant and had a lower inhibition of Omicron replication. All compounds inhibited SARS-CoV-2-MCherry-S-Delta or SARS-CoV-2-MCherry-S-Omicron. Cell-cell spread decreased in all treatments. Virus became resistant to FAC4 and 8 after 5 passages in cell culture, recovering similar or higher titers to the control. NGS revealed the presence of deletions and mutations in M, N, and E proteins. **Conclusion:** FACs inhibited SARS-CoV-2 replication with a post-entry activity, probably by interaction with structural proteins.

A331

Enterococci faecal phageome of preterm infants

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Abstract

Preterm infants (<32 weeks of gestational age) are at risk of developing pathologies such as necrotizing-enterocolitis (NEC) and late-on sepsis (LOS). Development of a stable gut microbiota in early life plays an important role in child's health and development. Enterococcus faecalis and Enterococcus faecium are the predominant within the enterococcus species, associated with the healthy and NEC microbiome in preterm infants. The preterm gut, like other environments contains bacteriophages relating to these species, but little is known about their role in the environmental fitness in the preterm gut environment. We here provide insights on enterococcal temperate phage genetic diversity and evolution within the preterm infants born at the Newcastle, Royal Victoria Infirmary neonatal intensive care unit. We present data on temperate phage carriage from 198 metagenomic samples of preterm infant stool, that present high relative abundance of Enterococcus (> 50%). These were processed through in-house bioinformatic pipeline to predict prophage regions within the metagenome assembled genomes. Prediction of these prophage regions and access to the preterm stool samples make this study a unique opportunity for phage isolation and characterisation. Novel panel of bacteriophages or bacterial lysogens with defined roles in the preterm infant gut may promote the establishment of a healthy gut microbiota in this high-risk cohort.

A332

Characterising Prophage diversity and function in the preterm infant pathogen, Staphylococcus epidermidis.

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Abstract

Staphylococcus epidermidis is a leading opportunistic pathogen due to its ability to form biofilms on indwelling medical devices. MLST analysis of *S. epidermidis* has identified strain type (ST) 2 and 5 as more important in human infections and carry a broader range of antimicrobial resistance genes. *S. epidermidis* is one of the most predominant bacteria found in human breast milk and has emerged as a leading pathogen in late onset sepsis in preterm infants, which contributes to the mortality and morbidity of neonates. The aim is to characterise *S. epidermidis* colonising early preterm infants and understand the role of temperate phages in early gut microbiome development in infants colonised with *S. epidermidis*. Stool samples taken from very preterm infants (<32 weeks gestation) from the NICU in the RVI, Newcastle upon Tyne in the first week of life, were sequenced using the Illumina HiSeq X10 2x150bp chemistry. The data was quality trimmed, host signature removed, and the microbial data assembled using megahit. Contigs were binned and GTDB-Tk was used for bacterial classification of the bins. MLST analysis enabled the identification of 11 different ST's, including ST2 and ST5. Prophage regions were predicted and analysed for AMR, virulence determinants and auxiliary genes. Our data demonstrates that *S. epidermidis* STs colonising the preterm infant gut harbour a diverse range of prophage regions. We hypothesise that prophage induction during antimicrobial stress in *S. epidermidis* will drive the evolution of this organism and alter the role it plays in the gut community which warrants further investigation.

A333

Development of *E. coli* production strains for safe bacteriophage preparations

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Abstract

Bacteriophage (phage) are an alternative or adjunct treatment to antibiotics as their lytic lifecycles can be harnessed to eradicate an infecting bacterial population, irrespective of MDR status. As phage are biological entities they need to be produced in a bacterial strain, this presents problems of potential carry-over of bacterial genomic content and the presence of lipopolysaccharide (endotoxin) on the Gram-negative cell wall, eliciting a human immune response to the therapeutic. To enable the production of safer phage preparations we are developing a set of host *E. coli* strains capable of amplifying a broad spectrum of phage active on clinical *E. coli* strains. We have screened hundreds of isolates from human and canine UTIs to identify strains which are generally susceptible to phage and then further selected two isolates from these with minimal phage immunity systems, prophage content and antibiotic resistance genes. Lipopolysaccharide pathways in the two strains are currently being modified to remove acyl chains and therefore reduce the level of downstream processing required. In addition, the receptors of phage that do (81/100 phage tested) and do not infect (19/100 phage tested) this pair of strains are being identified with the intention of adding phage receptors to the propagation strains to increase their utility. The aim is to produce two strains capable of amplifying a large proportion of coliphage with sequenced phage preparations that should require minimal further processing before their therapeutic application.

A334

Understanding the drivers of phage-resistance of uropathogenic *E. coli* ST131 in urine

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Abstract

Urinary tract infections are a common cause of antibiotic prescription in humans and companion animals. The majority are caused by uropathogenic *Escherichia coli* (UPEC), from which the clonal group ST131 is of particular concern due to the multi-drug resistance and high pathogenicity that characterise the strains in this group. Phage therapy is regarded as a promising alternative to antibiotics that could potentially be used to treat ST131 infections. However, there are still issues that need to be addressed to prevent failure of the treatment due to phage-resistance. For instance, we show in this work that phage susceptibility depends on the culture medium and the nutrient availability; phage-sensitivity tests could therefore be misleading if only standard laboratory media is used. Using *E. coli* strain EC958, a well characterised member of the ST131 clonal group, we have dissected the cause of phage-resistance in artificial urine when predated on by a single phage, LUC4. The escape population was analysed at different time points by re-challenging with the phage. We observed two different mechanisms playing a role: (1) fixed resistance caused by an extensive array of mutations in the surface receptor and associated regulators, and (2) increasing resistance with population density that we attribute to metabolic changes that allow the bacteria to replicate, but not the phage. Importantly, the sensitivity could be re-instated by nutritional supplementation. This means that the phage activity could be enhanced if these are delivered with the appropriate nutrients directly into the bladder.

A335

DEVELOPMENT AND VALIDATION OF MAYARO VIRUS NANOLUCIFERASE AS A POTENT TOOL FOR DRUG DISCOVERY

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Abstract

Mayaro virus (MAYV) has drawn the attention for its ability to cause febrile illnesses, clinical complications, and disabling chronic manifestations. Furthermore, there is no licensed antiviral therapy to treat Mayaro fever. In this context, novel tools to the development and validation of antiviral against MAYV represent an important approach for drug discovery. Here we assessed the antiviral activity of the aminoadamantane (1), an adamantane derivative, against MAYV *in vitro*, and also validated its antiviral action against Zika virus (ZIKV) in order to assess its potential broad-spectrum activity. A stable infectious clone of MAYV harboring the nanoluciferase reporter under the CMV promoter (MAYV-*nanoluc*) was developed based on the strain MAYV BeAr20290. For antiviral assays, Vero-E6 cells were treated with (1) in a two-fold serial dilution ranging from 2 to 400µM and infected with MAYV-*nanoluc* at a multiplicity of infection (MOI) of 0.1 for 24h, or with $ZIKV_{PE243}$ at an MOI of 0.01 for 72 hours. MAYV replication levels were quantified by measuring *nanoluciferase* activity, and ZIKV titers by focus formation units using immunofluorescence assay. The results demonstrated that (1) presented a dose-dependent activity, with selectivity index (SI) of 2.3 for MAYV and 2.42 for ZIKV. Our findings demonstrate the applicability of the MAYV infectious clone as a powerful tool for high-throughput antiviral screening aiming to identify drug candidates against MAYV. Additionally, we identified aminoadamantane (1) as an interesting derivative to be repurposed for the treatment of Mayaro and Zika fevers, which may further stimulate its potential as a broad-spectrum antiviral drug.

A336

Development and testing of a diagnostic pipeline for phage therapy of canine urinary tract infections.

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Abstract

Urinary tract infections (UTIs) are very common in dogs, with Escherichia coli being the most frequently isolated bacterial species. Dogs with other conditions such as diabetes, kidney disease and hyperadrenocorticism also have high rates of UTI (up to 50%) and some infections fail to respond to antibiotic treatment and become chronic, with animals suffering long term painful infections and, in some circumstances, requiring euthanasia. Infected urine is also hazardous, exposing owners to multi-drug resistant bacterial pathogens. Bacteriophages (phages) are viruses that specifically kill bacteria, but a key challenge is to identify the best phages to treat each infection. We have recently generated a bacteria-phage database based on >9000 interactions and built predictive models to select phages for treatment based on the genome sequence of an infecting strain. We are now using the OmniLog (Biolog) to perform interaction screening to identify more “broader-spectrum” phage to extend the number of phages with predictive models so that we can offer bespoke cocktail preparations for the majority of E. coli UTIs presenting at the Small Animal Hospital, University of Edinburgh. Currently, using MinION sequencing technology on DNA directly extracted from a clinical urine sample we can use the models to select phage predicted to be active to combine in cocktails. We will initially test these predicted cocktails on infected urine samples from canine patients with a further objective to study the efficacy and impact of the bespoke phage treatments when combined with currently prescribed antibiotics. The prediction pipeline can provide data on both selection of phage and antibiotic. Overall, this research is intended to bring adjunct phage therapy for canine UTIs closer to the clinic to alleviate animal suffering.

A337

ANTI-SARS-COV-2 ACTIVITY OF AN AMAZONIAN NATURAL COMPOUND

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Abstract

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) emerged in late 2019, and currently there is no approved specific antiviral treatment. Amazonian biodiversity represents an immeasurable source of natural compounds with potentially potent antiviral activities. In this context, LFA is a glycosyl flavonoid that was predicted to be able to inhibit the SARS-CoV-2 proteins $3CL^{pro}$ and RdRp by *in silico* analysis. The aim of this work was to evaluate the *in vitro* anti-SARS-CoV-2 activity of LFA. The antiviral activity of LFA was first assessed using a vesicular stomatitis virus (VSV) chimera expressing the reporter eGFP and the S protein of SARS-CoV-2 (VSV-eGFP-SARS-CoV-2-S) to infect Vero cells and investigate the inhibition of virus entry. Cell viability analysis was performed in parallel. Then, the LFA anti-SARS-CoV-2 activity was validated by infecting Vero cells with SARS-CoV-2_{WT} (Wuhan strain) and adding LFA at non-cytotoxic concentration in different stages of virus infection. Inhibition of infection was indirectly measured by cell viability under infection and treatment, using MTT assay.

LFA inhibited 70% of VSV-eGFP-SARS-CoV-2-S infection at a non-cytotoxic concentration, with a selectivity index (ratio between cell viability and virus infectivity) of 18:1, suggesting a potent inhibition of viral entry. In SARS-CoV-2_{WT} infected Vero cells, the results corroborated the previous *in silico* data, showing up to 61,2% inhibition of SARS-CoV-2 replication. LFA possesses a great potential as an antiviral drug candidate and could also be useful as a template to the development of novel antiviral drugs against SARS-CoV-2.

A339

Human norovirus viral particles: bacteria-associated production and application

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Abstract

Being a causative agent of acute gastroenteritis in all age groups human norovirus (NoV) is a serious threat to the public health. No approved vaccines against norovirus are currently available due to the challenges that affect anti-NoV vaccine production: high antigenic diversity of the virus and it uncultivable nature. The virus-like particles (VLPs) based on the self-assembled major capsid protein VP1 have shown enormous potential in specific immune response modulation. Among the actual ways of NoV VP1 production based on genetic engineering, application of the prokaryotic systems has shown to be inefficient (VP1 was synthesized in an insoluble form) and the eukaryotic systems have failed to be cost-effective.

The construction of an efficient system for deriving the soluble form of NoV VP1 was the main purpose of our study.

The strategy for creation the VP1 synthesis system involved application of pColdI-based expression vectors with inserted genes encoding VP1 of NoV GI.3, GII.4 and GII.17 genovariants and selection *Escherichia coli* Arctic (DE3) as the recipient strain.

The cold shock induction of recombinant VP1 genes associated with the joint implementation of pColdI vector and low-temperature tolerant strain resulted in a notable increase in yield of the full-length VP1 NoV GI.3, GII.4, and GII.17. All the target proteins were synthesized in the soluble form.

The production capacity of the strains ranged from 10 to 99.8 mg per 1 litre of culture liquid. All the identified target proteins exhibited significant immunogenic properties crucial for the production of NoV-specific antibodies.

A340

DeepDrop: Deep learning and Droplet microfluidics for single cell level label free bacterial analysis

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Abstract

Droplet microfluidics manipulates and analyzes small volumes of fluids in microscopic droplets. This offers us precise control over volume and composition of these droplets and enables long term observation of encapsulated components. In this study, we harness the potential of object detection oriented deep learning and droplet microfluidics to study the growth and lysis of freely swimming individual bacterial cells within microfluidic droplets. Our method can detect individual cell division and lysis events purely based on cell morphology, without the requirement of any fluorescent labelling. We have already demonstrated that our method can be used to study the lysis of *E. coli* cells in presence of T7 bacteriophages. In this study, we implement this method to study individual and co-cultures of clinically relevant strains of *P. aeruginosa* and *S. aureus*. These bacterial strains often occur together in lung infections and might potentially have a synergy in combating the effects of antibiotics. We performed individual growth experiments to calculate the cell division time and room temperature as well as at 37°C. We then co-encapsulated both strains and observed their growth in microfluidic droplets. Cell division times in droplets were comparable to those in a turbidity-based assay. This confirms that our platform is capable of replicating assays at much smaller volumes. We will further use this platform to study the possibility of synergetic interaction in presence of phages that effect either strain.

A341

Structural characterisation of a phage tail-like bacteriocin from *Pseudomonas sp*. by cryo-Electron Microscopy

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Abstract

Antibiotic resistance is a global health crisis predicted to cause more deaths than cancer by 2050 and as a result, there is a current drive to not only search for new antibiotics, but to also investigate antibiotic alternatives. Bacteriophage therapy is routinely discussed as a possible alternative to antibiotics to treat bacterial infections, however a limitation to their use is the bacteriophage replication cycle, that relies on the bacteriophage infecting and replicating within the target bacteria and could result in the generation of mutations with undesirable consequences. Some bacteria have evolved to produce phage tail-like bacteriocins (PTLBs) which share many similar structural and genetic features to a bacteriophage with the principal difference that PTLBs lack a capsid and the genetic material contained within. As a result, PTLBs are not self-replicating and are therefore a strong contender as a therapy to treat bacterial infections as they can be titered. An additional strength of PTLBs is their high specificity and ability to target a particular bacterial strain. We have purified a previously uncharacterised PTLB from a *Pseudomonas* strain isolated from ground water and solved the structure by cryo-Electron Microscopy (cryo-EM). The structure has highlighted the lack of a tape-measure protein and the possible implications this may have to the structure: function relationship of PTLBs.

A343

Modelling the dual impact of phage and antibiotics on *in vitro* MRSA population dynamics

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Abstract

Antibiotics are the first line of defence against Methicillin-resistant *Staphylococcus aureus* (MRSA), yet resistance to all antibiotic classes has been observed across MRSA populations. Adding phage to therapies could overcome antibiotic resistance (ABR), however horizontal transfer of ABR genes by phage via generalised transduction has been found to occur between MRSA with high rates. Cross-disciplinary solutions are needed to untangle and quantify these complex dynamics, to determine how antibiotic-phage combination therapies could be delivered effectively.

We develop a novel multidisciplinary approach to study the impact of erythromycin and tetracycline, alongside 80α phage, on two competing strains of MRSA, each harbouring a gene encoding resistance to either erythromycin or tetracycline. We co-culture the strains and measure their hourly concentrations over 24 hours after exposure to different concentrations of antibiotics and phage. To uncover the invisible microbiological interactions, we develop multiple mathematical models of bacteria-antibiotic-phage interactions and fit them to our data.

Without phage, increasing antibiotic concentrations lead to decline of the susceptible strain for the first 6-8 hours, unexpectedly followed by recovery to increasingly variable bacterial concentrations. Our models accounting for antibiotic degradation and metabolism capture observed dynamics better than simpler established models. With phage, the dynamics are additionally impacted by lysis and generalised transduction of ABR genes.

Mathematical models based on experimental data achieve greater accuracy in describing bacteria-antibiotic-phage dynamics, which is essential for informing interventions and understanding ABR development. Our methods also provide a framework for future investigations for more complex environments, *in vivo* systems, and other antibiotic-pathogen combinations.

A344

Title: Characterising the role and evolution of temperate bacteriophages in chronic respiratory infections of Pseudomonas aeruginosa in Cystic Fibrosis (CF) patients using their first isolate.

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Abstract

Cystic fibrosis (CF) is an autosomal-recessive genetic disease defined by reduced mucociliary clearance, continuous inflammation of the lung, and chronic bacterial infection. Pseudomonas aeruginosa (Pa) is the predominant bacterial pathogen in chronic infection in CF patients associated with morbidity and mortality. Temperate phage genomes integrate into the Pa host genome and introduce genes that contribute to the adaptation and evolution of Pa in the lung. The project aims to characterise temperate phages as potential markers of evolution focusing on how they alter bacterial cell physiology and what this could mean for the pathophysiology of the lung in CF patients.

We focus here on phage carriage in early CF infection and chronic colonisation. We compare at a genomic level isolate (n =333) from the Newcastle Pa collection highlighting the difference in bacteriophage carriage and whether the phages present are more representative of later lung infections in CF patients. The aim is to determine if there are key genes carried by phages that are aligned to early infection of the lung that may be important to focus more on mechanistic investigations. The work will be achieved with the use of multiple bioinformatic tools as well as molecular techniques. Furthermore, investigate the effects of phages on metabolomics, lipidomics, and gene expression when induced from the isolates using CRISPRi, for a more targeted approach. Moreover, characterise and compare the genomic and proteomic diversity of temperate phages in Pa isolates from the IPCD database. Overall, the project aims to establish that temperate phage provides selective advantages in Pa and this arms-race evolution contributes to the chronic infection in the CF lung.

A346

The role of prophages in *Citrobacter rodentium* pathogenicity

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Abstract

Temperate phages integrated as prophages in bacterial genomes constitute important vehicles for horizontal gene transfer and, thus, are a major source of new genes and functions for bacteria, such as virulence factors and antimicrobial resistance genes. *Citrobacter rodentium*, a natural mouse-restricted enteric pathogen, contains 10 prophages in its genome whose function remains largely unknown. To study the role of prophages in *C. rodentium* infection *in vivo*, we deleted all prophages from its genome (CRΔ10 strain). Similar to wild type *C. rodentium*, infection of C57Bl/6 mice with CRΔ10 resulted in mild self-limiting colitis. In contrast, C3H/HeNCrl mice, which succumb to wild type *C. rodentium* infection due to host and microbiome-associated factors, survived infection with CRΔ10. This suggests prophages contribute to *C. rodentium*virulence in the C3H/HeNCrl *in vivo* model. Further study of their roles is needed to identify the implicated mechanism/s and understand how prophages could have contributed to the adaptation of *C. rodentium* to the murine host environment and pathogenesis. This will provide new insights into the involvement of host-specific factors in susceptibility to bacterial infections.

A347

Isolation, characterisation and investigation of novel bacteriophages therapeutic potential.

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Abstract

Infectious bovine keratoconjunctivitis (IBK) is the most common ocular disease of cattle. IBK has a considerable negative impact on cattle welfare, and is a highly contagious disease, with outbreaks more common in the warmer months. IBK, commonly referred to as pink eye, is caused by *Moraxella bovis* and transmitted by the face fly (*Musca autumnalis*). IBK is characterised by four disease stages with varying degrees of corneal opacity, ulceration and, in severe cases, can lead to ocular rupture and blindness. Attempts have been made to vaccinate cattle against IBK, but data suggests a lack of efficacy.

Currently, antibiotic medication, applied topically or via injection, is the predominant IBK treatment. However, as antibiotic resistance is one of the biggest threats to our global health and food security, developing a more targeted therapeutic approach, using naturally occurring bacteriophage, would be of benefit to treat this difficult and debilitating disease.

This research involved the isolation of *Moraxella bovis* bacteriophages, characterisation of these phages using genome sequence analysis and transmission electron microscopy, and testing their potential as therapeutics. Novel *Moraxella bovis* bacteriophage, which resemble phage in the Myoviridae family, were isolated and characterised. A library of clinically relevant and geographically diverse strains of *Moraxella bovis* were used to determine the host range and therapeutic potential of these novel phage for use in the treatment of IBK.

A349

Antimicrobial activity of bacteriophage encoded protein gp28 against multi-drug resistant Pseudomonas aeruginosa

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Abstract

Introduction: *Pseudomonas aeruginosa* is a Gram-negative bacterial pathogen commonly associated with nosocomial infections. Treatment of *P. aeruginosa* infections is increasingly problematic due to biofilm formation and widespread antibiotic resistance. As a result there is an urgent need for novel treatments. This study aimed to determine if bacteriophage-derived antimicrobial peptide gp28 demonstrated activity against *P. aeruginosa.*

Methods: Antimicrobial susceptibility of *P. aeruginosa* was established for a range of relevant antibiotics using EUCAST disc diffusion methodology. The minimum inhibitory concentration of gp28 was determined using EUCAST broth dilution technique, at a range of concentrations between 40-150 mg/ml. Biofilms were formed in 96-well microtitre plates for 24 hours, before gp28 was added at various concentrations up to 100 mg/ml for 24 hours. Biofilm mass was measured using the crystal violet method. Additionally, biofilms were grown on glass cover slips and treated with 100 mg/ml gp28 before visualisation using SEM. The effect of gp28 in combination with tobramycin was determined using broth dilution and total viable counts.

Results: *P. aeruginosa* was resistant to all antibiotics tested. The MIC of gp28 was 100 mg/ml with significant biofilm disruption seen at 100 mg/ml (p=<0.05). A combination of gp28 and tobramycin at sub-inhibitory concentrations inhibited growth significantly more than either treatment alone (p=<0.05).

Conclusion: *P. aeruginosa* is a significant pathogen, and this work demonstrates that the antimicrobial peptide gp28 could have potential as a therapeutic agent against *P. aeruginosa*. Furthermore, it illustrates the potential for gp28 to be used in combination with other antimicrobial agents.

A350

Inhibition of HIV-1 Subtype C protease with gallotanin

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Abstract

High genetic diversity is a major contributory factor in the development of drug resistance, in addition to challenges in diagnosis and treatment monitoring in the therapeutics of human immunodeficiency virus (HIV). Within the wide HIV-1 diversity, differences in mutational frequency, disease progression, drug response and transmission amongst HIV-1 subtypes have been shown. In spite HIV-1 subtype C (HIV-1C) being the most prevalent variant globally, none of the available drugs nor screening assays for inhibitory molecules have been developed targeting the genetics of this important subtype. This study therefore aimed to overexpress and biophysically characterize HIV-1C protease to serve as a reagent in the development of assays for routine screening of molecules inhibitory to HIV-1C. Heterologous expression of HIV-1C protease isolates that are prevalent in South Africa was carried out in *Escherichia coli (E. coli* (BL21-DE3). The secondary and tertiary structures of the proteins were determined using, circular dichroism (CD) and fluorescence spectroscopy respectively. Thereafter, interaction studies to delineate interaction properties of gallotanin for possible inhibition of protease were conducted. Furthermore, *in silico* studies to determine binding interactions of gallotanin against HIV-1C protease were also conducted. The expressed HIV-1C protease from the globally prevalent HIV-1C was shown to be structurally and functionally intact for application in downstream HIV-1 inhibition assays. Binding studies on the other hand revealed molecular interaction of the expressed HIV-1C PR with gallotanin documenting the first intermolecular interactions of the two molecules, a confirmation for the inhibition of HIV-1 PR with gallotanin.

A352

Bacteriophages in Human Milk: Inter-relationship between the core community of phages and lipids in very preterm milk

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Abstract

Bacteriophages (or phages) are viruses that infect bacteria and are ubiquitous in nature alongside their cellular hosts. Phages in human milk (HM) may benefit infant health by preventing pathobiont overgrowth in the gastrointestinal tract (GIT), and the associations with lipids in HM may promote vertical transmission of phages from mothers to the infant GIT. We used shotgun metagenomics and untargeted lipidomics to compare phage and lipid profiles of 99 preterm HM samples expressed over the first 100 days of life. Significant changes in the phage richness and lipid compositions were observed between lactational weeks of preterm HM. Amongst the phage communities in preterm HM, Siphovirus and Podovirus (but not Myovirus) were found positively correlated with long chain length fatty acids. We therefore performed an in vitro assessment to determine whether lipids attenuate the infectivity of phages. A significant reduction in the infectivity of Siphovirus (but not Myovirus) with an increased concentration of a very long chain length fatty acid which supports the findings of our omics study. This is the first taxonomic report and in vitro examination of phage-lipid interactions in HM and highlights the importance of phage carriage in HM and in the early life microbiota development in preterm GIT. Our findings suggest an evolved strategy for phage carriage in HM that could potentially have a therapeutic benefit for diseases associated with an imbalanced GIT microbiome that can be fatal in extremely low birth weight very preterm infants.

A354

Filamentous phages alter *Aeromonas hydrophila* virulence

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Abstract

Filamentous bacteriophages establish chronic productive infection in bacteria, which implies phage persistence in the form of prophage or extrachromosomal element that does not kill bacterial host. The phages continuously replicate and extrude from bacterial cells, further infect new hosts and potentially change bacterial phenotypic characteristics. *Aeromonas hydrophila* is Gram-negative opportunistic pathogen of animals and humans. The impact of filamentous phage infection on this emerging pathogen has not yet been studied.

The aim of this study is to investigate differences between *A. hydrophila* strains before and after filamentous phage infection via testing certain bacterial properties *in vitro* and *in vivo*.

Two filamentous phages designated as Af3 and Af12 were used for infection of six *A. hydrophila* strains. Strains in which phage infection was confirmed by colony PCR were further examined for change in biofilm formation, hemolysis, growth kinetics and *in vivo* virulence using zebrafish larvae.

Two out of six *A. hydrophila* strains were successfully infected with filamentous bacteriophage. Biofilm production was enhanced notably in case of both strains after phage infection. However, their hemolytic activity decreased significantly. Filamentous phage infection considerably inhibited growth of one strain. *In vivo* investigations revealed a reduction in bacterial count and mortality in zebrafish larvae infected with bacterial strains bearing filamentous phage after 24 hours.

The differences observed in this study indicate that filamentous phages decrease *A. hydrophila* virulence, but favour phenotypic traits responsible for chronic infections.

A355

The heads and tails of *Salmonella* Infantis control with bacteriophage in the *Galleria mellonella in-vivo* model.

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Abstract

Salmonella spp. are a group of pathogens attributed to both human and animal disease with a range of symptomatic severity. *Salmonella* Infantis is now the fourth most abundant serovar in Europe in humans, with pESI-like megaplasmid positive strains displaying enhanced virulence and antimicrobial resistance strategies. The principal aim of this study was to isolate and characterize novel bacteriophage with activity against poultry-associated isolates of Infantis, and to use the *Galleria mellonella* model for *in vivo* assessment of their therapeutic potential. Following isolation, phage were characterized through classical methods (e.g. Efficiency of plating) and genome sequencing. Nine phage were isolated, with genome analysis showing all to be members of the *Drexlerviridae* family and predicted to exhibit temperate lifestyles. These phages were found to be stable during long-term storage at 4 °C, displayed distinct *Salmonella* host ranges and had the ability to form lysogens. *Salmonella* Infantis was found to be more virulent than *S.* Typhimurium in the *G. mellonella* model. During early phage therapy trials with Infantis, the introduction of temperate phage or a lytic control (Felix01) resulted in greater mortality than with *S.* Infantis alone, unlike when the same lytic phage was used in conjunction with *S*. Typhimurium. Potential differences in the LPS structure of Infantis altering *G. mellonella* immune response, which may result in increased Galleria mortality is hypothesized and the subject of continuing investigation. Overall, this study has shown that temperate phage can have *in vivo* lytic activity on their bacterial hosts, and that for some *Salmonella spp*. phage lysis can result in increased *G. mellonella* death.

A356

Beyond Antibiotics: Genome-prophage infection network analysis of *Streptococcus suis* hints at exploitable phage characteristics for synthetic bacteriophage therapy applications

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Abstract

Streptococcus suis poses a significant health threat in the global swine industry, primarily managed with antibiotics and unreliable vaccines. Causing arthritis, meningitis and even death in pigs, its zoonotic potential raises concerns about transmission and antimicrobial resistance in humans. This genetically diverse species, traditionally classified into >30 serotypes based on capsular polysaccharides, presents a potential target for control through bacteriophage therapy without exacerbating antimicrobial resistance.

Utilising 2,119 *S. suis* genomes from the BV-BRC database, we employed annotation tools Prokka, eggNOG emapper, PADLOC, and geNomad to functionally annotate bacterial/phage genes and characterise prophages. Pangenome analyses were performed using Roary, and Scoary was used to identify genes potentially associated with different geno/phenotypes. Genome networks were created using Sourmash to calculate Jaccard Index of k-mer frequencies in bacterial/prophage genomes, and communities (clusters of similar genomes) were identified using the weighted Louvain method.

Pangenomes revealed a relatively small core (<200 genes) for *S. suis*, emphasising its diversity. However, communities exhibited larger cores (>1,200). While the species core consisted largely of metabolism and information processing genes, communities were enriched in strain-specific genes such as cell exterior and defense mechanisms. Prophage-genome infection networks identified diverse infection motifs within/across communities, suggesting infection by certain phages is not limited to isolates of genetic homology.

These findings underscore the potential of network analyses to uncover commonalities among isolates and exploitable bacteriophage characteristics, paving the way for the development of synthetic phage-therapies tailored to capitalise on shared genetic traits, providing an avenue to control *S. suis* infections.

BLOCK A

Session : Virus Workshop: Translating knowledge - understanding and preventing disease

A357

Assessing the antiviral activity of spirotetronate natural compounds against respiratory viruses

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Abstract

The influenza A virus is a major cause of severe respiratory tract infection, which results in 200,000 hospitalisations per year, in the UK. Vaccines have been developed to control the spread of infection, but vaccine hesitancy and antigenic drift limit their effects. Patients hospitalised with severe infection are given antiviral treatments, however, the virus has developed resistant mutations against all classes of existing antivirals, rendering many ineffective. Therefore, there is a crucial need for new, effective treatments.

We aimed to investigate the antiviral activity of a tetronic acid macrolide called MM 46115 against the influenza virus. The natural product is produced by the soil bacterial species *Actinomadura pelletieri* and has previously been demonstrated to have antiviral activity against multiple respiratory viruses, including influenza A, parainfluenza and respiratory syncytial virus.

We confirmed these early findings using plaque assays with the influenza A virus strain A/WSN/33, which demonstrated an IC50 of 1.25 µg (2.804 µM). Further plaque assay and quantitative reverse transcription-PCR data from infections performed over 2 – 24 hours showed treatment with MM 46115 produced lower virus titres for up to 6 hours, compared to without treatment. Cell viability assays were also performed to investigate the cytotoxicity levels of MM 46115. Addition of MM 46115 (12.5 – 0.0125 µg/mL) did not significantly reduce cell viability compared to the untreated group, however, a higher concentration of 125 µg reduced cell viability to 80%.

Therefore, our data indicates that MM 46115 and potentially other related compounds, may show promise as future antivirals.

A358

Inhibition of Massachusetts serotype avian coronavirus infectious bronchitis virus by a homodimer single-domain antibody targeting the S1 domain of Spike protein.

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Abstract

Gammacoronavirus infectious bronchitis virus (IBV) is the causative agent of an economically important respiratory disease of poultry. Despite vaccination, the many serotypes of IBV cause significant production losses. IBV relies on spike (S) protein to facilitate virus entry into host cells, and it also accounts for high serotypical and antigenic variation. In our study we aimed to block the spike protein with camelid antibodies with a single variable domain, also known as nanobodies or VHHs. Llamas were immunized with the S1 subunit or full-length S protein of two diverse IBV serotypes, M41 and QX. Based on initial screening of 44 monomer VHHs, flexible linkers were applied to create homodimer and homotrimer constructs of two clones targeting the S1 domain. Growth kinetic assays, plaque assays and ex vivo trachea organ culture (TOC) assays of five IBV serotypes were employed in presence of one of the most suitable homodimer constructs (VHH2221). Results suggest that VHH2221 was most efficient against IBVs of the Massachusetts (Mass) serotype including both the pathogenic M41 strain and attenuated strains Beaudeutte and H120. VHH2221 could prevent the reduction of tracheal ciliary activity in Mass serotype infected TOCs ex vivo. Dose-dependent reduction in viral titre and plaque size was also observed in primary chicken cells in vitro. Interestingly, VHH2221 had moderate or no effect on replication of representatives of 4/91, QX, Arkansas and D212 serotypes suggesting a serotype dependant mechanism of action. VVHs may provide an alternative serotype specific therapeutic for the control of IBV infections.

A359

Exploratory Study on Efficacy and Safety of a Visible Blue Light Device in Patients with Upper Respiratory Viral Infection

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Abstract

This study aims to investigate the therapeutic potential of blue light irradiation for managing common cold symptoms, targeting infected mucosae using a specialized device. The potent toxicity of blue light irradiation on target tissues was examined using the Comet assay, known to assess DNA damage in cells. The clinical study was designed as a randomized, double-blind, sham-controlled, exploratory study using the investigational device as a treatment for outpatients with a common cold. The study enrolled 20 subjects with at least 4 symptoms such as nasal obstruction/nasal discharge, sneezing, sore throat, and cough at baseline. Subjects were divided into study or control groups and treated a total of 2-3 times daily, a total of 9 times for 3-4 days. The primary outcome measure was evaluated with total symptom score improvement. In this study, blue light irradiation up to 20 J/cm^2 showed no genotoxicity in Vero cells. Additionally, significant symptom relief was shown in the total study group and virus-positive patients in the study group (p=0.036 and 0.017), compared to the control. Sub-analysis of the Ct value of qPCR for virus-positive patients showed a significantly different change in the study group (p=0.013). No local application site reaction and device-induced side events were reported. This research demonstrates promising outcomes, with blue light showcasing its potential as a therapeutic intervention for the common cold. The findings suggest a notable reduction in symptoms and a substantial impact on inhibiting viral replication.

A360

Production of enterovirus A71 genotype C4 VLPs in *Pichia pastoris*

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Abstract

Enterovirus A71 (EVA71) is a major causative agent of hand, foot and mouth disease (HFMD), often resulting in high fever and potential neurological complications. HFMD affects infants and young children, primarily across the Asia-Pacific region. During EVA71 infection, particles lacking viral genome termed empty capsids (ECs) are formed. These are composed of 60 copies of three structural proteins, VP0, VP3 and VP1 arranged on an icosahedral lattice. ECs are initially antigenically indistinguishable from virions, but are antigenically unstable and rapidly expand at moderate temperatures. ECs expressed in heterologous systems, in the absence of viral genome, are known as virus-like particles (VLPs). The absence of genome makes these VLPs non-infectious and suitable as vaccine candidates.

Previously, stabilising mutations have been selected in EVA71 genotype B2, which increased the antigenic stability of particles generated. Whilst several expression systems have been used to produce recombinant EVA71 VLPs, the yeast *Pichia pastoris* is a well-established technology in large scale biopharmaceutical manufacturing and currently are employed in licensed VLP-based vaccine for hepatitis B virus (HBV) and human papillomavirus (HPV).

The aim of this project is to express EVA71 (genogroup C4) VLPs in the yeast *Pichia pastoris*. We will characterise these VLPs using a combination of sedimentation gradient, western blot, ELISA, and antigen conversion assays to determine the extent of particle formation and stabilisation. The generation of these EVA71 VLPs in a yeast system may allow the development of a pipeline for the production of highly immunogenic antigenically stable EVA71 VLPs with potential applications for vaccine manufacture.

A361

Understanding enterovirus capsid assembly and maturation through the characterisation of a highly conserved capsid motif

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Abstract

Non-polio enteroviruses (EVs) are a significant threat to global health with between 10 to 15 million infections reported annually in the U.S. alone, at an estimated cost of billions of dollars to public health systems worldwide. This highly prevalent genus of viruses cause a variety of diseases in both humans and animals, ranging in severity from mild febrile illness to fatal paralysis and neurological complications such as meningitis and brainstem encephalitis. Despite the global importance of EVs, details of the pathways by which these viruses assemble and mature are still poorly understood.

To address this lack of understanding, we employed an *in silico* approach to predict residues essential for EV particle assembly and maturation. We identified a highly conserved motif and characterised the functional role of these residues in EVA71 maturation, as a representative EV model. Utilising mutant viruses, recovered from *in vitro* transcribed RNA, we assessed the effect of these mutations on particle maturation and infectivity and performed serial passage to assess phenotypic reversion. In parallel, we determined genome packaging and the sedimentation profile of each mutant virus. Together, this information has enabled us to identify and trap a number of intermediate EV particle states, helping to delineate the stepwise assembly and maturation of EV capsids. Moreover, this data has allowed us to identify critical interactions which control genome release during EV infection. This understanding will be further developed with structural studies and utilised to direct and guide the design of pan-antiviral compounds against enteroviruses.

A362

A lyophilised influenza HA1-HA18 pseudotype virus library for immunogenicity readouts of pan-influenza vaccines and therapeutics in LMIC facilities

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Abstract

With the rapid expansion of new universal influenza vaccine technologies, and requirements for enhanced surveillance capacity, there is an acute need to match this with immunogenicity readouts against all influenza A subtypes, especially in LMIC regions where many endemics occur.

A comprehensive influenza hemagglutinin (HA) pseudotype library encompassing Influenza A subtypes HA1-18 has been developed using lentiviral vector platform technologies and co-expressed proteases. These HA1-18 pseudotype viruses have been evaluated in influenza pseudotype microneutralization (pMN) assays using MHRA-NIBSC antibody reference sera and bnMAbs.

The pMN is highly sensitive and specific for detecting HA-specific neutralizing antibodies against influenza viruses and can be used to safely assess antibody functionality in vitro and with a wide choice of reporters. Once the neutralisation profile of each HA pseudotype was determined, aliquots were lyophilised, and subsequently re-constituted and re-neutralised, demonstrating that their antigenic structure remains intact.

Our HA assay platform will facilitate surveillance efforts and preclinical studies involving (universal, pan-subtype) immunization dosing regimens in LMIC where cold-chain shipping and access to -80C freezers are not available. This library is the most comprehensive available globally and can be harnessed to meet strategic objectives that contribute to the strengthening of global influenza surveillance, expansion of seasonal and zoonotic influenza prevention and control policies, and strengthening pandemic preparedness and response.

A363

Modular sample-to-sequence workflows to enhance genomic surveillance of Dengue virus in the Philippines

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Abstract

Dengue is a significant arboviral infection causing a wide range of clinical manifestations affecting more than 100 million people annually. The disease is a leading cause of morbidity in the **Philippines**, a country having one of the largest dengue disease burdens in Southeast Asia. With an overarching goal of enhancing existing surveillance platforms and leveraging on the increased availability of portable sequencers, we aim to optimize sample-to-sequence approaches for the accurate detection and genotypic characterization of DENV. Here we present benchmarking results of different workflows – an amplicon-based method **(DENV tiling)**, a semi-agnostic probe-based viral enrichment approach **(Twist TE)**, and two metagenomics workflows (**KAPA** and **SMART-9N**). Optimized protocols are tested on clinical samples from the Philippines and sequenced with a MinION device. Both DENV tiling and Twist TE generated near full-length dengue genomes on positive samples. DENV tiling offers a sensitive and low-cost option for active surveillance in outbreak settings. Primer specificity however limits its capacity to detect highly divergent strains or correct identification of other viruses with similar symptoms. The semi-agnostic and metagenomics workflows essentially address these challenges having the potential to capture dengue diversity at the onset of an outbreak to inform primer design. The unbiased metagenomics methods work optimally on samples with high viral load, while probe-based enrichment has sensitivity comparable to DENV tiling. Whilst mainly tested on DENV in the Philippines, all protocols can be further optimized to work with other viruses, and the portability of the MinION reinforces adaptability of these workflows by other endemic countries.

A364

The impact of COVID-19 on eye movements in persons with Severe Speech and Physical Impairment

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Abstract

Whilst there have been significant follow up studies on the effects of COVID-19, the impact of the disease on the neurological functioning in persons with Severe Speech and Physical Impairment (SSPI) has seen limited reporting. Quantitative assessment of video-based eye tracking is a simple, non-invasive means to map cognitive functions to specific brain circuits. Eyegaze is a robust alternative response modality for cognitive-linguistic assessment by persons with SSPI which traditionally rely on verbal responses or that require physical responses such as pointing.

A cued attention prosaccade task, using a VT3 Mini Eye Tracker and analysed using MangoldVision software, was carried out with an SSPI group and a neurotypical (NT) control group. Participants were grouped according to historical SARS-CoV-2 infection. We hypothesized that individuals who previously had COVID-19 would perform significantly worse than those who had not.

The mean time to first fixation (TTF) to the location of an abrupt onset peripheral target was significantly faster in the NT group than the SSPI group ($p<0.05$), as could be expected when comparing a neurotypical group to persons with neurological disorders. Unexpectedly, however, we found that the TTF was faster in both the NT Group ($P<0.001$) and SSPI Group ($P<0.01$) in persons who previously had COVID-19.

Using eye tracking it appears that COVID-19 (a) influences brain circuits that drive eye movements in reaction to visual stimuli, supporting reports that SARS-CoV-2 can disrupt neurotransmitter balance and amplify signals between neurons; and (b) does not differentiate between a pre-existing neurological movement impairment or lack thereof.

A365

Using dual pseudo- Micro-Neutralisaition (pMN) technology to distinguish SARS-CoV-2 and influenza neutralising antibody responses.

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Abstract

Respiratory viruses have been in the forefront of the public's mind since the start of the SARS-CoV-2 pandemic. The annual influenza season has not diminished following global lockdowns and so this has prompted the need to be vaccinated for both viruses on an annual basis.

Distinguishing neutralising antibody levels against each virus is critical for the evaluation of vaccines delivered simultaneously, usually in different arms, or combined vaccines currently in development. Additionally, determining responses in individuals with non-specific respiratory symptoms could be useful in a pharmacy setting. Current estimates put dual infections in approximately 2.5% of Covid-19 patients. Using a dual sero-assay reduces the amount of serum and time required to carry out these assessments.

Taking advantage of existing pseudotype assays developed by us, we present herein a dual neutralisation assay which utilises 2 different luciferase (Renilla and Firefly) reporter lentiviral vectors. We configured assays with various combinations of SARS-CoV-2 and influenza subtype/variant combinations and compared sensitivity of this dual approach to neutralisation levels seen in single virus pMN in a double blinded test of monoclonal antibody cocktails and then with a pre-screened serum panel. As we have taken a pseudovirus based approach there is the potential to tailor to specific vaccination candidates or currently circulating strains of each virus.

The use of this versatile assay could form part of the toolbox of analysis of dual vaccination candidates or an in house pharmacy test to determine treatment for mild respiratory infections.

A366

Randomized controlled trial of molnupiravir SARS-CoV-2 viral and antibody response in at-risk adult outpatients

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Abstract

Treatment of SARS-CoV-2 with the nucleoside analogue molnupiravir was reported to reduce viral load, hospitalisation and mortality in unvaccinated participants with early COVID-19 in the MOVeOUT trial. Based on these data, molnupiravir received emergency use authorisation in the UK in November 2021 for early treatment of SARS-CoV-2 in individuals at higher risk of complications.

Molnupiravir is metabolised intracellularly to NHC-triphosphate, which competes with natural cytidine and uridine for incorporation by the viral RNA-dependent RNA polymerase (RdRp) into the nascent viral RNA, leading to an increase of transition mutations. Lethal mutagenesis resulting from treatment eventually leads to viral extinction. However, the risk that some highly mutated viruses might remain viable and capable of onward transmission has been postulated.

Participants within 5 days of SARS-CoV-2 symptom onset were randomised to receive molnupiravir or Usual Care in the PANORAMIC virology sub-study. Molnupiravir accelerated viral load decline, but mostly failed to clear virus by the end of the 5-day course. By Day 14, molnupiravir treatment was associated with significantly higher viral persistence and significantly lower anti-SARS-CoV-2 spike antibody titres compared to Usual Care. Whole genome sequence of 1436 samples revealed increased transition nucleotide mutagenesis with molnupiravir treatment. Persistence of detectable viral RNA at Day 14 in the molnupiravir group was associated with higher numbers of transition mutations following treatment cessation. Our study indicates that 5-days of molnupiravir could drive the emergence of SARS-CoV-2, thus longer treatment courses should be tested to reduce the risk of potentially transmissible molnupiravir-mutated variants being generated.

A367

High-throughput screening of FDA-approved drug library using recombinant Cedar virus platform for *Henipavirus* antiviral discovery

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Abstract

Nipah virus (NiV) and Hendra virus (HeV) are highly pathogenic bat-borne paramyxoviruses belonging to the *Henipavirus* genus that can cause severe and often fatal respiratory and neurological disease in humans and livestock. Currently, an equine vaccine (Equivac®) against HeV is used in horses in Australia, yet there still remains no approved NiV or HeV vaccines or therapeutics for humans.

In an attempt to develop henipavirus-targeted antivirals, a high-throughput screening platform was previously established utilising a recombinant version of Cedar virus (rCedV), which is a non-pathogenic *Henipavirus* closely related to NiV and HeV. rCedV encoding a firefly luciferase gene (rCedV-Luc) was generated for use in high-throughput antiviral compound screening. This rCedV reporter is a valuable tool that is representative of *Henipavirus* infection that can be used as a surrogate for NiV and HeV infection but at CL-2.

Utilising this previously characterised system, we are screening an FDA-approved compound library consisting of over 2,700 licensed compounds that cover common targets whilst representing extensive and prominent research areas such as infection, immunity, and nervous system diseases. The use of FDA-approved compounds for antiviral screening is highly advantageous as drug repurposing offers a less time-consuming and more streamlined approach to antiviral drug development. Ultimately, this work aims to identify antiviral compounds against *Henipavirus* infection that can be further developed for therapeutic use in humans.

A368

The antiviral efficacy of interferon-β1a (SNG001) against different SARS-CoV-2 variants, in vitro.

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Abstract

With the emergence of SARS-CoV-2 variants resistant to monoclonal antibody therapies and limited global access to therapeutics, the evaluation of novel therapeutics to prevent progression to severe COVID-19 remains a critical need. Inhaled interferon-β1a (IFN- β1a) (SNG001) is being investigated as a potential treatment for severe respiratory viral infections including COVID-19. IFN-β1a is a cytokine that is naturally produced in the lungs, in response to viral infections. Sometimes invading viruses switch off this mechanism, to enable viral replication allowing the virus to spread. Administering aerosolised IFN-β1a directly into the lung airways allows for IFN-β1 levels to be restored at the site of infection and 'switches on' antiviral defences. Inhaled IFN-β1a treatment is being developed as a potential broad-spectrum variant agnostic treatment for severe viral lung infections including those caused by SARS-CoV-2.

In this study we tested the antiviral efficacy of IFN-β1a, *in vitro*, against a variety of SARS-CoV-2 variants to provide reassurance that IFN-β1a was effective against the different variants as they emerged. A 96-well micro-inhibitory, foci reduction assay with VeroE6 cells was used to determine the amount of IFN-β1a (IU/ml) causing a 50% reduction (IC_{50}) in viral foci compared to the virus only control.

Results indicate that the in vitro efficacy of IFN-β1a is retained against different SARS-CoV-2 variants including Alpha, Beta, Delta, Gamma and Omicron BA.2 variants with IC_{50}'s ranging from 2.35 to 14.10 IU/ml. Here we discuss the importance of continued monitoring of the efficacy of novel and licensed treatments for COVID-19 and preparedness for future pandemics.

A369

Identification of pathogens associated with respiratory disease in Albanian cattle

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Abstract

Identification of the pathogens causing respiratory disease in Albanian cattle would help veterinarians control, prevent and treat cases of respiratory illness in this country. Currently, there appears to be limited sequence information on pathogens of Albanian cattle so combining surveillance with sequencing would enable comparison of infectious agents in this country with the rest of Europe and the World. A trained veterinarian collected 85 nasal pharyngeal and pulmonary samples from cattle without or with symptoms of respiratory disease. Protocols were developed to extract viral nucleic acids and detect three viral pathogens associated with the bovine respiratory disease complex by PCR. Targeted sequencing of the envelope proteins expressed by Bovine coronavirus and Bovine respiratory syncytial virus enabled phylogenetic analysis to compare Albanian clinical isolates with those from the rest of the World. As expected, the Bovine coronavirus isolates were most closely related to other clinical isolates from across Europe. Only a small proportion of the cattle with respiratory disease were positive for the viral pathogens screened in this study, and some healthy cattle were positive for these pathogens, suggesting aetiological agents not included in this study contribute to the bovine respiratory disease complex in Albania.

A370

Examination of Gene Evolution and Phylodynamics in Marek's Disease Virus

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Abstract

Marek's disease virus (MDV) is an alphaherpesvirus causing an economically-important oncogenic and lymphotrophic disease in chickens. Vaccination has been the predominant control method since 1970 and whilst vaccines prevent symptoms, viral infection and transmission continue at lower levels. Such non-sterilising vaccines may promote evolution towards increased virulence. We examined imperfect vaccine-induced MDV evolution and transmission as part of the Ecology and Evolution of Infectious Diseases (EEID) USA-UK consortium.

MCMC time-correlated trees were inferred for complete genomes and *meq*, stratified by location and pathotype. Field strains were deep-sequenced and reads were subjected to reference-based assembly. Re-mapping was performed against consensus sequences to obtain minor variants.

The substitution rate of *meq* was 10-fold greater than the full genome rate. Geographical and pathotype stratification was evident for both datasets. Whereas the full genome remained stable over time, the population of *meq* expanded over a 20-year period then declined. The number of variants was greatest in the large tegument protein, *meq* and *ICP4* genes.

The *meq* oncogene is a major MDV virulence factor. Although it evolved more rapidly than the overall MDV genome, its diversity peaked in 1997 and subsequently declined. Additionally, no positively selection was detected in *meq*. Together this suggests *meq* may have reached its evolutionary limits. Minor haplotypes were identified in known virulence genes as well as other genome regions. Mixed viral populations have previously been described in large DNA herpesviruses. They simulate, to a lesser extent, the diversity within RNA viral quasispecies and may similarly potentiate DNA virus evolution.

A371

Structural and functional characterisation of the SARS-CoV-2 Envelope ion channel

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Abstract

SARS-CoV-2 poses a continued risk to global health, placing vulnerable populations at a sustained, elevated risk of severe disease and mortality. Moreover, the risk of both acute and longer term sequelae remains in the general population due to unprecedented viral variation.

Novel antivirals against SARS-CoV-2 are required, ideally with drug targets outside conventional enzymatic targets. The ion channel produced by the SARS-CoV-2 Envelope protein (E) represents an attractive potential drug target, as it acts as a major virulence factor during multiple life cycle stages. Moreover, two mutations observed in recent Omicron variants support a key role for E during transmission and/or pathogenesis.

Both structural and functional ambiguity surrounds oligomeric E complexes, even extending to channel stoichiometry. Lipid-borne structures lack the complete protein sequence, whereas the micellar models that exist also raise concerns. To address this, we have compared both C-terminally truncated peptides and full-length recombinant protein through a variety of assays, incorporating the T11A and T9I Omicron polymorphisms.

We will describe analysis comparing lipid and micelle-associated E using native and cross-linking SDS PAGE, as well as electron microscopy, defining both protein- and membrane/membrane mimetic-dependent effects upon channel stoichiometry. In parallel, channel gating, ion specificity and drug sensitivity are assessed using indirect *in vitro* assays alongside electrophysiological techniques. Observations from these systems inform more relevant structural analyses for lipid-borne full-length protein, suited to bespoke antiviral development.

A372

Production and optimisation of Crimean-Congo haemorrhagic fever virus pseudotyped virus and its application in neutralisation assays

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Abstract

Crimean-Congo haemorrhagic fever (CCHF) was first described in 1944 and the virus identified 25 years later. CCHF virus (CCHFV) triggers outbreaks, with a 10-40% fatality rate. Endemic to Africa, the Balkans, Middle East and Asia, it carries potential for broader geographical expansion. No licenced human vaccines or therapeutics exist. Most vaccines efficacy assays involve infectious CCHFV, a biohazard level 4 pathogen. A safer alternative is the use of CCHFV pseudotyped virus (PV). The codon-optimised sequence of CCHFV glycoproteins GnGc (strain IbAr10200) was synthesised and cloned into an expression plasmid and two different PV production systems were compared using a vesicular stomatitis virus (VSV) and a human immunodeficiency virus (HIV) core. Only the VSV vector led to the successful production of CCHFV PV with a yield of 4.72 x 10^4 TCID50/mL, whereas the use of a HIV vector did not produce a usable titre of CCHFV PV. Adjusting the amount of CCHF GnGc gene expression plasmid and transfection reagents failed to significantly alter the yield of infectious pseudotyped virus. The CCHFV/VSV PV was then applied for the development of a neutralisation test. A known neutralising monoclonal antibody anti CCHFV-Gc, 11E7, and a panel of seven convalescent sera, were all able to neutralise CCHFV PV in a dose response manner. More samples are being sourced to qualify the assay and the next steps will be to compare the results with those from a traditional neutralisation test to establish whether the use of CCHFV PV is a feasible alternative to the authentic virus.

A375

Evaluating the neutralising activity of the 1st WHO International Standard for anti-Chikungunya virus IgG using two pseudotype systems expressing Chikungunya virus glycoproteins.

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Abstract

Chikungunya virus (CHIKV) can cause a febrile illness and long-term complications such as arthralgia, resulting in significant economic impact. Currently only one CHIKV vaccine is licenced for use in the US and new vaccines are currently in development. Rigorous testing is required to establish an immunological correlate of protection for CHIKV. Pseudotyped viruses have been extensively used as a surrogate to measure neutralising activity against high-hazard viruses and provides an alternative method for laboratories lacking high containment. Furthermore, for many systems, it has been demonstrated that neutralisation of the pseudovirus correlates with that of an authentic virus.

CHIKV glycoproteins were therefore expressed in two different pseudotype systems using (1) a vesicular stomatitis virus or (2) lentiviral core, both incorporating a luciferase reporter gene. The potency of the 1st WHO International Standard (IS) for anti-CHIKV IgG (1502/19) was similar in both CHIKV pseudotype systems and appeared to be greater than the potency achieved in CHIKV neutralisation assays. These results are comparable with data from the WHO collaborative study that established the 1st WHO IS for anti-CHIKV IgG. These initial results indicate that using pseudotype systems expressing CHIKV glycoproteins may be able to provide an alternate avenue to authentic anti-CHIKV serological responses. We are currently applying pseudovirus based assays to establish a correlate of protection conferred with experimental vaccines in model systems.

A377

Metagenomics pipeline comparisons and their application to Preeclampsia

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Abstract

Metagenomics can identify unknown pathogens through indiscriminatory sequencing of host and microbial, viral, and fungal species. We compared metagenomic methods for detecting pathogens using a high-host background mock community and validated on clinical tissue and blood samples. We did this by evaluating untargeted short-read Illumina and long-read Oxford Nanopore Technology and a targeted viral panel. Further, we compared commonly used metagenomic classifiers and analysis methods. We find that at high viral loads, Nanopore and Illumina non-targeted workflows have similar sensitivity and specificity, with Nanopore having quicker turnaround for small sample sizes. At lower viral loads, Nanopore requires increased sequencing depth than Illumina to reach the same sensitivity. Robust thresholds for classifiers aid in standardising the analysis.

One application of the above is the study of severe Preeclampsia (PE), a gestational disease ultimately organ dysfunction. The placenta likely plays a central role in the pathophysiology of PE due to abnormal spiral artery modelling, however, the underlying molecular basis is not understood. We have applied metagenomic methods to clinical samples of preeclamptic cases and controls, as well as known-infected patients, to establish whether infection is associated with novel immune signatures in PE patients identified through single cell and spatial transcriptomics of the placenta, or simply a potential confounding factor. We thus aim to advance the study of PE.

A378

Use of Ionising Radiation for the Development of Vaccines Against Zika Virus and Respiratory Syncytial Virus

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Abstract

Zika Virus (ZIKV) and Respiratory Syncytial Virus (RSV) are enveloped RNA viruses with various challenges for developing vaccines. ZIKV causes diseases including Congenital Zika Syndrome, resulting in microcephaly in babies if contracted by pregnant women, causing a 14.3x increase in infant mortality rate. RSV causes 3.6 million hospital admissions and 100,000-200,000 deaths per year in under 5-year-olds. Two vaccines against RSV are licensed and there are currently no approved vaccines against ZIKV.

Inactivated vaccines relying on chemicals can damage protein-based antigens needed for protective immune responses. Ionising radiation has been understudied as a method of inactivation due to its past expensiveness. However, radiation can damage viral RNA without destroying key protective antigens and is thus an interesting alternative inactivation method.

This project aims to inactivate ZIKV and RSV using various modalities of ionising radiation to develop effective vaccines for both viruses.

ZIKV and RSV were exposed to varying doses of gamma radiation
to determine the optimal inactivation doses. Effective radiation doses were ascertained by viral titration and RT-qPCR. To determine if radiation impacted virus structure, cryo-EM was performed.

Data indicates that both viruses are inactivated at a radiation dose of 14-18kGy. Preservation of protein expression and conformation was confirmed by western blot for RSV's pre-F protein and ZIKV's E protein. Confocal microscopy showed RSV F-protein attaching to Hep2 cells, suggesting natural conformation after irradiation.

In conclusion, ZIKV and RSV can be inactivated by gamma radiation. Future work will focus on in vivo experiments to determine immunogenicity and functionality in mice.

A379

A complete molecular and cellular toolkit for working with seasonal human coronavirus OC43

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Abstract

The COVID-19 pandemic accelerated coronavirus research — however many fundamental areas of coronavirus biology remain unaddressed, in part due to practical challenges: working with coronaviruses in higher containment, growing viruses to high titres, and the ability to manipulate viruses through reverse genetics.
We present a complete toolkit for seasonal human coronavirus OC43 (HCoV-OC43), the closest betacoronavirus to SARS-CoV-2 circulating in the human population. We optimised culture and plaque assay conditions to routinely yield HCoV-OC43 stocks at titres greater than 10^8 pfu/ml, permitting infections of human cell lines.
Further, we developed ancillary assays to facilitate study of HCoV-OC43, including viral genome sequencing via tiling amplicons, RT-qPCR primer sets, western blotting, immunoprecipitation, and immunofluorescence.
Additionally, we developed a novel reverse genetics system that bypasses the need for commonly-used yeast-based assembly, in vitro transcription, or polymerase extension reactions. We constructed a fluorescent HCoV-OC43 clone, and confirmed successful virus rescue 3 days post chemical transfection of cultured cells with the target DNA construct.
Lastly, using these tools with the Crick's High Throughput Live Virus Neutralisation platform and sera from participants in the UCLH/Crick Legacy Study (NCT04750356), we detected neutralising antibodies in 282/282 (100%) of analysed participants. Combined with our finding of susceptibility to widely-available antivirals, these data support continued classification of HCoV-OC43 as a Hazard Group 2 pathogen by ACDP.
Overall, this work lays the foundation for the use of HCoV-OC43 as a tractable, relevant, and biosafe model for the study of a wide range of molecular and virological aspects of betacoronaviruses.

BLOCK A

Session : Virus Workshop: Virus interaction with the host organism and implications for pathogenesis

A380

Challenges in Imaging Analyses of Biomolecular Condensates in Cells Infected with Influenza A Virus

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Abstract

Biomolecular condensates are crucial compartments within cells, relying on their material properties for function. They form and persist through weak, transient interactions, often undetectable by classical biochemical approaches. Hence, microscopy-based techniques have been the most reliable methods to detail the molecular mechanisms controlling their formation, material properties, and alterations, including dissolution or phase transitions. However, technical challenges in microscopy-based analysis persist. This paper discusses imaging, data acquisition, and analytical methodologies' advantages, challenges, and limitations in determining biophysical parameters explaining biomolecular condensate formation, dissolution, and phase transitions. In addition, we mention how machine learning is increasingly important for efficient image analysis, teaching programs what a condensate should resemble, aiding in the correlation and interpretation of information from diverse data sources. Influenza A virus (IAV) forms liquid viral inclusions in the infected cell cytosol that serve as model biomolecular condensates for this study. Our previous work on IAV inclusions was established using a framework involving fixed and live cell imaging to measure inclusion dynamics. This subsequent technical paper, which explores how different modalities in data acquisition and processing impact the robustness of results to detect bona fide phase transitions by measuring thermodynamic traits in fixed cells. Using solely this approach would greatly simplify screening pipelines. For this, we tested how single focal plane images, Z-projections, or volumetric analyses of images stained with antibodies or live tagged proteins altered the quantification of thermodynamic measurements. Customizing methodologies for different biomolecular condensates through advanced bioimaging significantly contributes to biological research and potential therapeutic advancements.

BLOCK B

Session : Finding the needle in the haystack: microbial surveillance in complex samples

A381

High-throughput SARS-CoV-2 antiviral testing method using Plate-Based Image Cytometer

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Abstract

The COVID-19 pandemic has created a worldwide public health crisis that has since resulted in 6.96 million reported deaths. The pandemic prompted the immediate response of researchers around the world to engage in rapid vaccine development, surveillance programs, and antiviral testing, which resulted in the delivery of multiple vaccines and repurposed antiviral drug candidates. However, the emergence of new highly transmissible SARS-CoV-2 variants has renewed the desire for discovering new antiviral drug candidates with high efficacy against the emerging variants of concern. Traditional antiviral testing methods employ the plaque-reduction neutralization tests (PRNTs), plaque assays, or RT-PCR analysis, but each assay can be tedious and time-consuming, requiring 2-3 days to complete the initial antiviral assay in biologically relevant cells, and then 3-4 days to visualize and count plaques in Vero cells, or to complete cell extractions and PCR analysis. In this work, we developed a high-throughput antiviral testing method employing the Celigo Image Cytometer to investigate the efficacy of antiviral drug candidates on SARS-CoV-2 infectivity using a fluorescent reporter virus by measuring the cytotoxicity effects on the healthy host cell line. Compared to traditional methods, the assays defined here eliminated on average 3-4 days from our standard processing time for antiviral testing. Moreover, we were able to utilize human cell lines directly that are not typically amenable to PRNT or plaque assays. Image cytometry can provide an efficient and robust method to rapidly identify potential antiviral drugs to effectively combat the rapidly spreading SARS-CoV-2 virus and its variants during the pandemic.

A382

Beyond Mutation: Understanding Molecular Evolution Across Viral Species

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Abstract

Background

We aim to create a taxonomy of quantitative differences in parameters of evolutionary variation across viruses, in terms of evolution speed (substitution rate) and evolution type (selection pressure).

Methods

All viral GenBank sequences, containing coding sequences and collection dates, were parsed using **biopython**, and aligned to their reference sequences using **Needle**. Multiple sequence alignment was performed using **MAFFT**. Non-coding regions of genomes were removed.

Recombination analysis was performed using **Simplot**. Clock like behaviour was measured using **Tempest**. Substitution rate was measured using **BEAST**. Selection analysis was performed using **SLR**.

Results

The above methods produced 393 high quality alignments of less than 50kb with 10 to 80 sequences and at least 3-5 years of collection date range.

Selection Analysis 30% of alignments didn't show any selected sites and for the remainder, 2137 positively selected sites were present.

Molecular clock of the 60% of alignments that showed no recombination, 63% showed correlation coefficient > 0.5 in TempEst, indicating clocklike behaviour.

Substitution rate for those alignments with R > 0.5, substitution rates divide evolution speed to five categories.

Conclusion

Segmented viruses behave differently in evolutionary parameters mentioned in results.

Significant variability seen in recombination, molecular clock and substitution rate patterns studied within analysed viral data sets suggests that they all may not serve as a consistent taxonomical markers.

On taxonomical distribution for positive selection, two families exhibited opposing selection patterns.

Non-synonomus/ synonymous substitution ratio varies among selected proteins functions.

A383

Genomic Epidemiology of SARS-CoV-2 in Norfolk, UK, March 2020 – December 2022

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Abstract

Background: In the UK, the COVID-19 Genomics UK Consortium (COG-UK) established real-time national genomic surveillance for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) during the COVID-19 pandemic. As a COG-UK partner, Quadram Institute Bioscience sequenced over 60,000 SARS-CoV-2 genomes, contributing to Norfolk becoming the most densely sequenced region in the UK. Retrospective SARS-CoV-2 lineage dynamics investigation in this region will allow for understanding of the pandemic's development and variant diversity.

Methods: 29,406 SARS-CoV-2 whole genome sequences and accompanying metadata from Norfolk were extracted from the COG-UK dataset, dated between March 2020 and December 2022, representing 9.9% of regional COVID-19 cases. Sequences were lineage typed using Pangolin, and subsequent lineage analysis carried out in R.

Results: 401 unique global lineages were identified, with 280 appearing more than once and 125 ten times or more. 92.0% of sequences were a variant of concern (VOC), with Alpha, Delta, and Omicron accounting for 8.6%, 34.9% and 48.5% of sequences respectively. The remaining lineages largely appeared in the early pandemic prior to VOC designation and were labelled as 'pre-VOC' lineages. Four epidemic waves were identified and composed of pre-VOC, Alpha, Delta, and Omicron lineages that spanned the period of study.

Conclusion: This study is the first to assess SARS-CoV-2 diversity in Norfolk across a large timescale within the COVID-19 pandemic. SARS-CoV-2 was both highly diverse and dynamic in its evolution in Norfolk between March 2020 – December 2022, with a strong VOC presence. The study highlights the utility of incorporating genomic methods into pandemic response.

A384

Characterisation of the s2m RNA structure in the Avian Coronavirus Infectious Bronchitis Virus.

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Abstract

A hairpin stem loop secondary RNA structure, known as the s2m, is present in the genomic 3' untranslated region (UTR) of a range of positive sense RNA viruses, including Picornaviruses, Astroviruses, and Coronaviruses. Deletion of the s2m in the *Betacoronavirus* SARS-CoV-2 does not appear to affect replication or pathogenicity either *in vitro* or *in vivo*. However, this is not the case for the *Gammacoronavirus* Infectious Bronchitis Virus (IBV); our previous research suggests the s2m is not a requisite for *in vitro* replication, but is implicated in viral fitness in an *ex vivo* tracheal organ culture system.

Using reverse genetics, we altered the canonical structure of the s2m in different IBV strains, representing different genotypes and clinical pathogenicity, and assessed the impact of these changes on RNA structure and viral processes. Selective 2'-Hydroxyl Acylation analysed by Primer Extension (SHAPE) Mapping was utilised in order to characterise the s2m altered variants and assess the effect of s2m disruption on RNA structure. Furthermore, alteration of the s2m leads to changes in growth kinetics in *ex vivo* tracheal organ culture for some strains, but not others; no differences in replication were observed *in vitro*. This may suggest a strain specific role for the s2m in IBV.

A385

Replacement of the SARS-CoV-2 ORF3a gene with either mNeonGreen or mScarlet attenuates viral replication and innate immune evasion in vitro.

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Abstract

The SARS-CoV-2 genome encodes at least nine accessory proteins, including the putative viroporin ORF3a. The ORF3a protein plays a role in many stages of the viral replication cycle, including acting as an immune modulator. To investigate the role of ORF3a, we generated two recombinant SARS-CoV-2 viruses in which the ORF3a gene was replaced with either mScarlet (SARS-CoV-2-Δ3a-mS) or mNeonGreen (SARS-CoV-2-Δ3a-mNG). These viruses have an ancestral (Wuhan-Hu-1) backbone with a D614G Spike (SARS-CoV-2-S-D614G). The resulting viruses generated a fluorescent signal after infection in both A549-ACE-2-TMPRSS2 (AAT) and VeroE6-TMPRSS2 (VTN) cells. The SARS-CoV-2-Δ3a-mNG virus was found to be genetically stable in AAT and VTN cells, but the mScarlet gene was deleted during SARS-CoV-2-Δ3a-mS virus passaging in VTN cells, creating SARS-CoV-2-Δ3a-ΔmS. The SARS-CoV-2-Δ3a-mNG, SARS-CoV-2-Δ3a-mS and SARS-CoV-2-Δ3a-ΔmS viruses all replicated to a lower titre and produced smaller plaques than SARS-CoV-2-S-D614G. Interestingly, the additional deletion of mScarlet provided a replication advantage compared to SARS-CoV-2-Δ3a-mS, as the SARS-CoV-2-Δ3a-ΔmS virus produced higher virus titres and larger plaque sizes. In comparison to rSARS-CoV-2-S-D614G, the SARS-CoV-2-Δ3a-mS and SARS-CoV-2-Δ3a-mNG viruses were found to be more sensitive to pre-treatment of cells with type I interferons and did not exhibit a dose dependent increase in replication in the presence of the JAK-STAT pathway inhibitor, Ruxolitinib. Currently, the viral and cellular transcriptomes from SARS-CoV-2-Δ3a-mS and SARS-CoV-2-Δ3a-mNG infected AAT cells are being investigated to determine differences in comparison to SARS-CoV-2-S-D614G. In conclusion, replacement of the ORF3a coding sequence has impacted the replication of SARS-CoV-2 and its evasion of the innate immune response *in vitro*.

A386

Uncovering Allelic Associations and Geographical Patterns in the Cytomegalovirus genome

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Abstract

Human cytomegalovirus (HCMV) is the most common infectious cause of congenital disability and causes fatal disease in immunocompromised individuals. High variability between HCMV genomes, frequent reinfection, and recombination pose considerable barriers to the development of vaccines. To characterise the evolutionary landscape and better inform vaccines' development, we used mathematical modelling to delineate variable regions. We identified 74 multiallelic regions (2-8 alleles). Thirty-two regions, together with the conserved part of the genome (86%), showed geographic structure, while 42 regions showed similar distributions of alleles across populations and enrichment for immunomodulatory functions.

To understand the biology of multiallelic regions we explored allele co-inheritance. Our findings reveal distinct geographical haplotypes in co-inherited regions 26 and 27 (non-coding) and in UL82 (pp71) and UL86 (capsid protein) corresponding to regions 36 to 40. African and European strains exhibit different haplotype frequencies. Remarkably, the most common European haplotypes were absent from African sequences, suggesting either a founder effect or geographically related selection pressures. In regions with non-geographically segregating alleles, co-inheritance occurs, particularly in glycoprotein-coding genes like UL55 (gB, regions 22-24) and UL74/UL75 (gL and gH, regions 28, 30, and 31).

Our approach had several advantages over using the entire genome, as it increased the statistical power by focusing on the most variable regions. Overall, our findings contribute to the understanding of the evolutionary pattern of HCMV and may aid in the vaccine's development.

A387

Measuring cytotoxicity in cells infected with African swine fever virus

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Abstract

African swine fever virus (ASFV) is a large complex DNA virus that causes African swine fever, a haemorrhagic disease of pigs with up to 100% mortality. Due to the virus' size and complexity, the functions of many of the proteins remain unknown. *In vitro* infection of cells with ASFV usually results in cell death within 24 hours. *In vivo* other factors, such as anti-ASFV antibody functions, may influence the extent of cell death or the rate at which these cells die. To determine how best to investigate these functions, a variety of cytotoxicity assays were compared for their use to measure cytotoxicity in ASFV-infected cells under laboratory conditions. Here we show that two assays that require transport of components across the cell membrane fail to detect death of both lab-cultured cells and purified porcine bone marrow cells infected with an attenuated ASFV isolate, however an assay based on changes of the external cell surface detect death successfully in both cell lines. This result suggests ASFV-infected cells undergo membrane changes that interfere with the transport of these components across the cell membrane. This has important implications for the development of assays based on cytotoxicity, such as measuring antibody functions, which are important for better understanding the B-cell response against ASFV and any protective, or detrimental, effects this may have.

A388

Superinfection exclusion is a potent barrier to SARS-CoV-2 coinfection

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Abstract

The ability of viruses to coinfect individual cells is regulated by superinfection exclusion (SIE), a mechanism whereby a virus blocks secondary infection by genetically similar viruses. Coinfection allows the generation of novel viral strains by genetic exchange, as happened repeatedly in the history of coronaviruses such as severe acute respiratory syndrome 2 virus (SARS-CoV-2). SIE should limit the opportunities for this but, despite the importance of genetic exchange in SARS-CoV-2 evolution, SIE has not yet been described for any coronavirus. Here, we use fluorescent reporter viruses and flow cytometry to demonstrate for the first time that SARS-CoV-2 coinfection of individual cells is limited by SIE, indicating that SIE is a overlooked barrier to the generation of recombinant SARS-CoV-2 variants. We then showed that influenza A viruses (IAV) can infect individual cells in which SARS-CoV-2 has established SIE, and vice versa, showing that SIE is a selective barrier to homologous coinfection. In a plaque assay setting, we did observe interference between SARS-CoV-2 and IAV during multicycle infection, as both viruses failed to spread in the presence of each other. This effect was abrogated by treatment with ruxolitinib, indicating the involvement of the type 1 interferon response. Together, our data show that SIE is an important factor in restricting coinfection and hence genetic exchange between SARS-CoV-2 strains, even though it does not prevent SARS-CoV-2 from establishing coinfections with other respiratory viruses.

A389

Molecular Detection of Newcastle Disease and Avian Influenza Viruses in Exotic Bird Species in Indonesian National Bird Park

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Abstract

Newcastle Disease virus (NDV) and Avian Influenza virus (AIV) are globally widespread and have caused infections with a wide range of clinical manifestations in different species of birds. Although cases of asymptomatic birds have been reported from zoos and pets, NDV and AIV infections in peacocks, pheasants, cockatoos, and owls are often associated with neurological symptoms. Since mid-2020, information regarding contagious and fatal neurologic disorders has surfaced, and some cases were recorded in Taman Mini Indonesia Indah (TMII) Bird Clinic. Haemorrhages in the tracheal syrinx, lungs, and brain were observed. Due to the endemicity of NDV and AIV in Indonesia, molecular techniques were carried out to detect the presence of these viruses. Possible oropharyngeal and cloacal swabs and pathological organs from four asymptomatic and symptomatic species of birds were tested. Despite clinical and pathological presentation, gel-based and real-time reverse-transcription polymerase chain reaction (RT-PCR) did not detect NDV and AIV. Further investigation using species, genus, or family-specific primers need to be carried out to identify other potential causative pathogen.

A390

miMic - a novel multi-layer statistical test for microbiome disease

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Abstract

Background Differential abundance analysis is at the core of microbiome statistical analysis. However, microbial data presents three prominent challenges: The microbiome data is sparse and most species are absent from most samples. The microbial abundances vary across different scales. There are inner relations between different taxa, reflecting the taxonomic structure. A microbiome statistical test should handle these three challenges.

Results. Here, we introduce miMic (Mann-Whitney iMage Microbiome), a straightforward yet remarkably versatile and scalable approach that effectively addresses compositional effects and inherent taxonomic relationships. miMic consists of three main steps: data preprocessing and translation to a cladogram of means, an apriori nested ANOVA to detect overall microbiome-condition (label) relations, and a post hoc miMic test along the cladogram trajectories. We propose a novel metric for an unbiased comparison of methods in the case of missing ground truth and show that miMic drastically decreases the False positive rate while preserving the total positive rate. Using an analytical test case, simulations, and real-world examples, we demonstrate the accuracy of miMic compared to existing methods.

Conclusions. While microbiome data is often very high dimensional, and as such suffers from a multiple measurement problem when compared with a phenotype, the taxonomic structure can be used to mitigate this limitation and ensure a high discovery rate with a very low false discovery rate.

A391

Oncomodulatory Potential of the Human Cytomegalovirus IE1 and pp65 Proteins

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Abstract

Human cytomegalovirus (HCMV) is a common pathogen that causes damage and disease in unborn children and immunocompromised individuals. HCMV has been implicated in the initiation and progression of cancer, and the virus has been detected in several types of cancer, including glioblastoma and breast tumours. The HCMV IE1 and pp65 proteins are frequently found in HCMV-associated cancers and are known to antagonize innate immunity, alter chromatin structure, dysregulate transcription, and inhibit tumour suppressors. We sought to determine whether HCMV promotes cancer progression, a process also known as oncomodulation, via the IE1 and pp65 proteins. Low-malignant MCF-7 breast cancer cells were transduced with IE1 and/or pp65 using lentiviruses and assessed for signs of increased malignancy. Malignancy was analyzed by growth curves, scratch assays, RT-qPCR of malignancy markers (ER, PR, HER2+, Ki67+), and RNA-seq. Despite continuous expression of IE1 and/or pp65, no consistent and statistically significant changes in cell proliferation, cell migration or expression of malignancy markers were observed. The RNA-seq data are still being analysed and will be presented as well. These results indicate that IE1 and pp65 may have a subtle if any, oncomodulatory role in breast cancer and suggest that other viral proteins may be required for oncomodulation by HCMV.

A392

The pig as a model to evaluate monoclonal antibody delivery platforms against influenza A viruses

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Abstract

With multiple antigenically distinct subtypes and propensity for mutation, the prevention and treatment of Influenza A virus infection remains a significant burden on public health. This has driven the desire to produce a universal influenza treatment that can efficiently and cheaply protect enough of the population to prevent the occurrence of future threats like the 1918 Spanish flu and 2009 swine flu pandemics.

Delivery of mRNA encoded monoclonal antibodies (mAbs) could be a powerful therapy for humans and animals. We have established a robust pig influenza model to assess the efficacy of mAbs delivery platforms. To evaluate their efficacy, a pharmacokinetic (PK) study of two recombinant anti-influenza mAbs was performed in pigs. The strongly neutralising human anti-haemagglutinin mAb, 2-12C, has been shown to reduce viral load and lung pathology in pigs when administered prophylactically at 15 mg/kg prior to challenge with 2009-pandemic H1N1 influenza. Due to the likelihood of pigs producing an anti-human response to 2-12C, a recombinant pig anti-haemagglutinin monoclonal antibody, pb27, was generated. Similarly to 2-12C, prophylactic administration of pb27 to pigs abolished lung viral load and pathology following H1N1pdm09 challenge. ELISAs were performed to quantify recombinant mAb concentration and assay anti-human responses in pigs dosed with human 2-12C. The functional activity of 2-12C and pb27 in serum was assessed using microneutralization. This study provided insight into clearance of recombinant antibodies given as a single dose to pigs and indicate utility of pigs as a model for assessing long term mAb delivery platforms against influenza A viruses.

A393

Describing the genomic and geographical structure of Human gammaherpesvirus 8

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Abstract

Human gammaherpesvirus 8 (HHV-8) is the aetiological agent of Kaposi's sarcoma. HHV-8 ORF-K1 and ORF-K15 have been reported to demonstrate significant variability, allowing HHV-8 categorisation into different genotypes. HHV-8 ORF-K1 studies showed six different clades (A-F) segregating geographically, whereas ORF-K15 is distinguished in three additional genotypes (P, M and N). However, these studies focused mainly on ORF-K1 and ORF-K15; knowledge of the rest of the genome remains limited. In this study, we utilised hidden Markov model (HMM) clustering to describe the variable regions across a dataset of 142 published HHV-8 whole genomes. HMM can determine the optimal number of alleles (or genotypes) that best explain HHV-8 genomic diversity. Our findings revealed that the HHV-8 genome is mostly mono-allelic (>99%) except for 10 multi-allelic regions each with 2 alleles. We found multi-allelic regions in ORF-K1 and ORF-K15 and identified regions in four additional genes – ORF7, LIR1, ORF47 and ORF56 – that are previously unreported. These genes encode transport proteins, glycoproteins, and DNA replication proteins. Geographical separation is observed in multi-allelic and mono-allelic HHV-8 regions, with a stronger distinction in multi-allelic areas. North American and European populations cluster together separately from African strains, which exhibit notably higher diversity than North American, European, and Asian populations. Since genetic differences between HHV-8 strains are associated with disease progression and variable regions are under immune selection, understanding the genomic diversity and the evolutionary processes that shaped this diversity could provide valuable information for the development of globally effective vaccines and help to identify novel drug targets.

A394

Modelling the emergence of a high-pathogenicity avian influenza virus (H7N7) outbreak among layers after initial incursion of the homologous low-pathogenicity avian influenza virus

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Abstract

H7N7 low-pathogenicity avian influenza virus (H7N7-LPAIV) incursions have preceded emergence of H7N7 high-pathogenicity (HP)AIV at several European layer hen outbreaks. Evidence from a UK layer H7N7-HPAIV outbreak informed *in vivo* modelling of the sequential events, beginning with H7N7-LPAIV precursor incursion.

Three groups of 17-week-old hens were inoculated (ocular-nasal) with three precursor H7N7-LPAIV candidates which featured: Group 1 - the classic LPAIV single-basic cleavage site (CS) (H7N7-SBCS); Group 2 - the dibasic CS (H7N7-DBCS) discovered at the outbreak (both represented direct H7N7-HPAIV precursors); Group 3 - a related European H7N7-LPAIV which possessed a DBCS within a haemagglutinin gene which was very similar with the H7N7-HPAIV's, but its other genetic segments differed.

In Groups 1-3, H7N7-LPAIV inoculations caused limited viral shedding and restricted H7-antibody responses, and no deaths, by 14-days post-inoculation. Subsequent H7N7-HPAIV challenge (including AIV naïve hens in Group 4) showed that prior H7N7-LPAIV inoculation protected against mortality to varying degrees: 100%, 87%, 75% and 37% in Groups 1-4, respectively (n=8 hens / challenge group). Protection did not affect overall H7N7-HPAIV shedding in all groups, nor systemic dissemination. The study finished at 14-days post-challenge, when surviving hens showed ongoing (or completed) H7N7-HPAIV clearance in all groups.

Emerging H7-specific humoral immunity following H7N7-LPAIV inoculation provided a likely mechanism for protection against H7N7-HPAIV mortality. However, other challenge survivors in Groups 1 and 2, which did not reveal any prior H7-specific serological reactivity, may have benefitted from another host-response mechanism against mortality, possibly involving the conserved internal viral genes among H7N7-SBCS, H7N7-DBCS and H7N7-HPAIV.

A395

Characterisation of Prophage in the Canine Oral Microbiome

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Abstract

Periodontal disease (PD) is a condition that features inflammation of the gingiva, bone, and periodontal ligament. PD is one of the most common conditions diagnosed in small animal veterinary practises. PD typically involves the accumulation of subgingival bacterial biofilms. Bacteriophages are viruses that infect bacteria, and prophage are bacteriophage genomes which integrate into the host genome. Prophage infection may play a key role in dynamics of microbial communities.

The aim of the study was to evaluate prophage infection in common species of the canine oral microbiome and to assess the rates and characteristics of prophage in PD pathogens.

From reviewing the literature, 62 canine commensals and pathogens were selected.

The 8184 assemblies were downloaded from Genbank. Viral contigs were identified using Phispy, and the resulting 9056 phage contigs were annotated with Pharokka.

On average, 40.5% of bacteria contained prophage. Bacteria most associated with health were the least likely to be infected at 30.2%. Antimicrobial-resistance (AMR) genes were present in 704 prophage, and related to 16 different drug classes. These AMR prophage infected 24.1% of the bacterial species. In addition to AMR genes, 14 classes of virulence-factor prophage genes were found in 9.6% of bacterial species. In conclusion, we have demonstrated that prophage have the potential to greatly impact the microbiome. Genes carried by prophage have the capability of influencing on the pathogenicity of the microbiome, influencing disease progression and treatment responses. Therefore to fully understand the microbiome within health and disease it is also necessary to consider the phageome.

A396

Comparison of the acute pathology of Chikungunya virus infection in New and Old World Non-Human Primates

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Abstract

Chikungunya (CHIKV) is a mosquito-borne RNA alphavirus that causes acute fever, rash, joint swelling and chronic severe arthralgia in humans. Old World non-human primates have been used as a laboratory model to characterise infection and study disease pathology. Following the introduction and explosive spread of Chikungunya virus in central and South America, we wished to explore the value of New World monkeys as a model system for studying infection and disease.

Thermoregulation in non-human primates exhibits a distinct diurnal pattern best studied by constant monitoring with remote telemetric devices. Constant wireless monitoring is achieved by the surgical implantation of devices which send a signal at set intervals to receivers and a laptop.

A group of 3 Red-Bellied Tamarins (Saguinus labiatus) were inoculated with Chikungunya virus by intra-dermal injection. Constant wireless monitoring of body temperature was undertaken with readings taken every 30-60 seconds with telemetry devices surgically implanted in the peritoneum. These data were compared with that from 10 cynomolgus macaques (Macaca fascicularis) in which identical devices had been implanted subcutaneously.

Disruption of thermoregulation was observed in both species following infection by comparison with the normal diurnal rhythm observed prior to infection. This disruption was characterised by a failure of body temperature to fall overnight. This would have not been detected with manual readings taken during normal working hours.

The location for the implant, chosen for animal welfare, had an impact on the quality of the data. Data recorded from intra-peritoneal implants were more reliable and provided a cleaner temperature profile.

A399

Serological evidence of non-notifiable low pathogenicity avian influenza virus in UK poultry employing a pseudotype based assay

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Abstract

Avian influenza viruses (AIVs) are named according to the composition of their envelope glycoproteins: haemagglutin (HA) and neuraminidase (NA). Sixteen different HA and nine different NA subtypes circulate in avian reservoirs globally. In the United Kingdom (UK), the H5 and H7 subtypes of AIV are notifiable avian diseases (NAD). However, non-notifiable AIVs also circulate, often asymptomatically through both wild and captive bird populations, and so serological evidence of infection can often be the only indicator of circulation. Here we sought to assess serological reactivity of sera taken from poultry as part of disease investigations looking for NAD, and screen them for evidence of antibodies to non-notifiable AIVs. Whilst the UK has undergone successive epizootic waves of high pathogenicity AIV (HPAIV), with high morbidity and mortality rates, LPAIVs also circulate, although they are usually sub-clinical or mild in clinical presentation. Sera from poultry collected between 2012-2023, that had previously tested negative for H5 and H7 specific antibodies, were screened for reactivity to non-notifiable AIVs. 45% of sera had antibodies to non-notifiable AIV. A panel of pseudotype viruses was generated and deployed harbouring a specific HA subtype antigen of those subtypes most commonly circulating in wild birds in the UK (H1, H3, H4, H6 and H11). Here we report on the serological detection rate of these LPAIV subtypes in UK poultry (6% for H3 and H4). This data provides greater detail of LPAIV subtypes which incur and are capable of infecting UK poultry, helping to inform risk of future AIV incursions.

A400

Mycoviruses in *Aspergillus fumigatus* and their effects on host sensitivity to antifungal drugs

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Abstract

Mycoviral infections have been shown to influence phenotypes of host fungi, including sensitivity to antifungal drugs. Mycoviruses in Aspergilli were first detected over 50 years ago; however, new mycoviruses are continuously being discovered. This investigation aims to study mycoviruses in *Aspergillus fumigatus*, causative agent of aspergillosis in immunocompromised individuals, and explore their effects on the host sensitivity to antifungal drugs. A collection of 42 *A. fumigatus* clinical isolates was screened for the presence of mycoviruses using a small double-stranded (ds) RNA extraction protocol and reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR). A low prevalence of mycoviruses in *A. fumigatus*, particularly members of the recently established family *Polymycoviridae*, was detected by small dsRNA extraction and confirmed by the more sensitive RT-qPCR. Concurrently, *A. fumigatus* virus-free (VF) and virus-infected (VI) isogenic lines were tested for drug sensitivity using an XTT metabolic assay in different growth media, including synthetic sputum medium (SSPM) that recapitulates the lung environment components. Differential sensitivity of VF and VI isogenic lines was observed against a range of antifungal drugs: VI *A. fumigatus* was shown to be more sensitive to nikkomycin Z but more resistant to amphotericin B as compared to VF. In both cases, the effects of mycovirus infection were more pronounced in SSPM. This investigation advances our knowledge in mycovirus prevalence and understanding of host-virus interactions in a medically important fungus.

A401

Investigation of Spike protein substitutions observed over a short period of time in SARS-CoV-2 infected immunocompetent individuals through functional cell-based assays

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Abstract

SARS-CoV-2 is a rapidly evolving beta-coronavirus responsible for the COVID-19 pandemic which has caused over 5.4 million deaths worldwide since its identification in 2019. Many genomic mutations in biologically important locations have characterised novel SARS-CoV-2 lineages causing global waves of the pandemic. Global genomic sequencing efforts have been able to monitor these outbreaks, track transmission clusters, and examine genomic evolution in real-time to help inform policy. Viral evolution is constant and random and important in determining viral infectiousness and validity of treatments to curb the pandemic. In this cluster of SARS-CoV-2 infected immunocompetent individuals in a closed transmission chain we investigated intra-host viral genomic evolution of both dominant and minor populations through NimaGen-Illumina sequencing. This longitudinal transmission study showed that rapid genomic mutation can occur in healthy individuals in the absence of immunosuppression, where it has been characterised previously. To examine the biological relevance of
these mutations, lentivirus pseudovirus particles containing Spike protein mutations found in these individuals were generated. Preliminary data from functional assays including receptor-binding assays and micro-virus neutralisation assays suggests that these mutations show phenotypic differences compared to the wild-type. Analysis is ongoing to determine the full extent of the differences. These assays are important tools to help elucidate any effects amino acid substitutions have on viral infection regarding receptor binding and immune-evasion in the host. Thus, they are imperative for future vaccine and therapeutics development and for monitoring viral evolution.

A402

Virus-mediated immunotherapy targeting Severe Acute Respiratory Syndrome Coronavirus 2

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Abstract

The COVID19 pandemic caused by SARS-CoV2 is propagated by unmitigated prevalence and evolution of the virus to escape from adaptive immunity. Nevertheless, subversion of innate immune responses plays an important role in both the establishment of infection as well as progression to severe COVID. Notably, delayed and downregulated responses to type I interferons (IFN) are integral to successful virus transmission.

Oncolytic viruses are an important mode of immunotherapy against cancer. However, we previously showed that type 3 Dearing strain oncolytic reovirus (*reo*), was able to target both malignant cells as well as underlying oncogenic virus infections, such as hepatitis C and B viruses, and Epstein Barr virus. Critically, this was due to rewiring existing innate immune evasive patterns and ensuing induction of a potent type I IFN response.

Reo has never reached a dose-limiting toxicity in cancer patients, displays pulmonary tropism, and is administered deep into the lungs via a nebuliser. Thus, we hypothesised that Reo may be a safe, effective way to deliver antiviral immunotherapy to the infected airway, avoiding the potential toxic effects of systemic/inhaled IFN.

Live and *uv*-inactivated Reo particles elicit antiviral responses in cell lines derived from alveolar and bronchial epithelia. However, tailoring agents leads to efficient induction of type I IFN and ISGs. We describe comparative therapeutic efficacy and characterisation of innate signalling in the context of SARS-CoV2 infection.

A403

The SARS-CoV-2 transmembrane accessory protein ORF7a promotes adhesion of activated T-lymphocytes to the endothelium

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Abstract

Introduction

SARS-CoV-2 ORF7a, a transmembrane accessory protein has 85% similarity with SARS-CoV ORF7a indicating conservation for functional purpose. SARS-CoV-2 ORF7a has been modelled *in-silico* and is proposed to interact with the metal ion dependent adhesion site in LFA-1, a T-lymphocyte receptor involved in adhesion to endothelial cells for transmigration. Questions therefore arise on if ORF7a has a function related to pathogenesis of COVID-19 in relation to the endothelium.

Methods

Recombinant SARS-CoV-2 ORF7a was incubated with endothelial cells *in-vitro* before T-lymphocytes were tagged with Calcein-AM and added to an endothelial monolayer as naïve cells or in the presence of 10ng/mL PMA and 1µg/mL Ionomycin. Adhesion of T-lymphocytes were quantitated by measuring fluorescence on a FLUOstar spectrophotometer at 485nm/525nm excitation/emission. Images were taken on an EVOS M7000.

Results

SARS-CoV-2 ORF7a did not increase adherence of naïve T-lymphocytes to the endothelium. T-lymphocytes incubated with 10ng/mL PMA; 1µg/mL Ionomycin; and 3pM ORF7a, increased their adherence to the endothelium by 12.9% ± 2.2%. Endothelial cells incubated with 3pM ORF7a also increased T-lymphocyte adherence by 10.2% ± 0.9%.

Conclusions

These results show evidence that SARS-CoV-2 ORF7a can promote activated T-lymphocyte adherence to the endothelium whilst having no effect on naïve T-lymphocytes. Promotion of extravasation may be a pathogenesis method by which SARS-CoV-2 increases vascular permeability and contributes to the cytokine storm seen in severe COVID-19.

A404

Investigating the Viral Characteristics of Virulent Systemic Feline Caliciviruses

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Abstract

Feline Calicivirus (FCV) is among the most common viruses to infect cats worldwide, with prevalence estimated to range from 10-90% depending on the population sampled. Typical FCV infection presents with oral ulcerations, fever and in some cases can also lead to symptoms such as pneumonia or 'limping syndrome'. However, some FCV strains have been isolated from cats exhibiting virulent systemic disease (presenting clinically with extensive mucosal and skin ulceration, oedema, and inner organ involvement), which can lead to high morbidity and mortality. Breakthrough VS-FCV infections have been recorded in vaccinated cats; therefore, there is considerable interest in developing novel therapeutics for use in the face of VS-FCV outbreaks. However, to design effective therapeutics, it is imperative that we better understand the viral characteristics that lead to virulent systemic disease.

Here, we used classical molecular virology techniques to investigate the differences between clinical isolates of FCV that were known to cause either acute respiratory or virulent systemic disease. Using *in vitro* cell culture, we assess the viral replication kinetics of avirulent and virulent strains, quantifying rates of viral translation and infectious titre. Subsequently, we resolved molecular structures of clinical isolates representative of strains associated with acute respiratory or virulent systemic disease, observing differences in the observed capsid flexibility. These findings provide further evidence that the viral capsid protein, VP1, might play a significant role in the pathogenesis of virulent systemic disease.

A405

Investigating the antibodies elicited by the IMOJEV vaccine

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Abstract

The genus *Orthoflavivirus* contains the most important arboviral pathogens of humans, several of which have caused large outbreaks in the 21st Century. As climate change affects Europe, we are likely to see greater disease burden. Japanese encephalitis virus (JEV), a *Orthoflavivirus* member, is the leading cause of encephalitis in Asia. Antibodies can be classified as either neutralising or non-neutralising. Only a fraction of the antibodies produced against flaviviruses neutralise the virus, yet the other functions of antibodies are under-studied. This study investigates both neutralising and non-neutralising antibodies, providing insight into the types elicited by the live attenuated JEV vaccine, IMOJEV. This was done by studying antibody binding, virus neutralisation, and Fc-mediator effector functions. Sera from patients in the FlaviPrime study (NCT03920111) showed that pre-exposure to natural *Orthoflavivirus* infection affected the type of antibody response. ELISAs with the JE virion (IMOJEV) or recombinant envelope protein were performed. Flavivirus-exposed participants had significantly higher binding to the recombinant envelope protein at 8- and 26-weeks post-vaccination, and to the JE virion at 26-weeks post-vaccination, compared to flavivirus-naïve participants. There were no correlations between live virus neutralisation and binding to either the recombinant envelope protein or IMOJEV in the flavivirus-naïve group and only weak correlations in the flavivirus-exposed group, indicating the presence of antibodies that bind, but do not neutralise. Further *in vitro* experiments are being conducted to elucidate on their ability to activate NK cells.

A406

Investigating the role of neutrophils in dengue virus (DENV) infection.

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Abstract

Four billion people are at risk of DENV infection, a flavivirus endemic in tropical and sub-tropical regions. Clinical severity varies substantially, from asymptomatic to fatal cases of Severe Dengue (SD). A primary feature of SD is increased endothelial permeability, resulting in plasma leakage. Neutrophils, the most numerous immune cells, contribute to vascular leakage in inflammatory diseases and varied disease severity in some viral diseases. This project aims to investigate the hypothesis that neutrophils are activated by DENV infection, and that in SD an elevated neutrophil response enhances endothelial permeability.

There is no evidence that DENV infects neutrophils, but several other myeloid cell-types are susceptible. To assess proteins released after DENV infection by activated myeloid cells, serum samples from patients with different clinical severities were interrogated using TMT mass spectrometry. Analysis of proteins annotated as “released by neutrophil granulation” or “enhanced in myeloid cell types” revealed >10 significantly elevated proteins in SD compared to mild disease. The possibility that DENV infects neutrophil precursor cells, or indirectly affects neutrophil production, was investigated *in vitro* by differentiating CD34+ hematopoietic stem cells along the neutrophil pathway and assessing proliferation, qPCR and cell surface marker profiles using flow cytometry.

Conclusions SD patients have elevated serum levels of neutrophil and myeloid lineage-linked proteins and DENV influenced neutrophil differentiation. Trans-endothelial electrical resistance assays will be used to assess whether selected proteins modulate neutrophil-induced endothelial permeability. Elucidation of the role of neutrophils in SD-associated endothelial dysfunction will improve understanding of how innate immunity influences responses to DENV.

A407

Impact of the SARS-CoV-2 Nsp1 mutations K164A and H165A on virus cytotoxicity and the production of stable replicon cell lines.

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Abstract

SARS-CoV-2 is a CL3 pathogen and establishment of a non-infectious viral surrogate system (termed replicon) for use at CL2 would broaden research access and facilitate antiviral testing. To achieve this aim, a series of replicons based on the Wuhan Hu-1 and Delta variant of concern genetic backgrounds were produced, using a yeast-based reverse genetic system. The Spike and Membrane genes were replaced with the puromycin N-acetyl transferase and *Renilla* luciferase genes respectively, to select stable replicon cell lines that have reporter enzyme activity. The ORF6 or ORF7a coding sequences were replaced with the fluorescent reporters; mNeonGreen or mScarlet. Furthermore, mutations were introduced into non-structural protein (Nsp) 1 (K164A/H165A) to reduce replicon cytotoxicity, reported previously for SARS-CoV. The replicons were replication competent and useful reporters as evaluated by luciferase activity, fluorescence, and single-molecule fluorescent *in-situ* RNA hybridisation. To establish stable replicon cell lines a panel of lines including VeroE6, BHK, A549, Caco-2, HEK293 and Huh7.5 were tested but puromycin selection failed to yield stable cell clones. To investigate whether the Nsp1 mutations in the replicons were showing the desired effect, a recombinant SARS-CoV-2 carrying the corresponding mutations was produced to investigate Nsp1 function in viral infection. Replication of the Nsp1 (K164A/H165A) virus was significantly attenuated in VeroE6-TMPRSS2, BEAS2-B-ACE2, Calu3 and A549-ACE2-TMPRSS2 cells. The mutations caused a sensitisation to interferons and a significant decrease in cytotoxicity. We have successfully generated replicons allowing the testing of antiviral compounds against SARS-CoV-2 at CL2 but were unable to derive stable replicon cell lines.

A408

Evolutionary dynamics and recombination in the avian coronavirus, infectious bronchitis virus.

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Abstract

Infectious bronchitis virus (IBV) is a gammacoronavirus causing respiratory illness in poultry resulting in significant economic losses. Vaccination is widespread, however the vaccines do not offer serotypic cross-protection, potentially imposing selective pressure directing the evolution of circulating viruses. Recombination is ubiquitous in IBV, preventing accurate inference of substitution rates. We aimed to utilise the Bayesian method “coalescent with recombination” to infer accurate substitution rates in IBV field isolates and investigate recombination. This study was undertaken within the Ecology and Evolution of Infectious Diseases (EEID) consortium investigating vaccine-induced evolution and transmission of viral pathogens of poultry.

The genetic sequence dataset comprising 365 IBV full genomes from field strains was down-sampled based on p-distances. The coalescent with recombination was implemented in BEAST2. For genotype GI-1, the non-structural 3b gene had the highest inferred substitution rate whereas the slowest was in spike subunit S2. The recombination rate was slower than the substitution rate for all genes. Per gene, recombination occurred in S1 more frequently than the other genes. Preliminary data suggests a similar range of substitution and recombination rates in the genes of other IBV major pathogenic genotypes and no substantial difference between genotypes regarding either the substitution rate or the recombination rate.

The coalescent with recombination method allowed for inference of substitution and recombination rates across different scales of the avian coronavirus IBV. We aim to confirm preliminary data and extend this study to other IBV genotypes of major clinical significance.

A409

Generation of porcine iPSC derived Hepatocyte Like Cells for the study of Hepatitis E Virus

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Abstract

Hepatitis E Virus (HEV) causes ~15,000 deaths and 44 million infections annually. However, the viral replication cycle is still being elucidated and antiviral strategies are mostly inexistent. HEV research is limited by the lack of efficient culture systems with slow, low titre virus growth.

Cancer-derived cell lines, while convenient for lab-adapted strains, contain disrupted metabolic, immune and apoptotic pathways, limiting their biological relevance. Primary human hepatocytes (PHHs) provide a more authentic culture system and can support clinical isolates; however, PHHs vary from donor to donor, may dedifferentiate or die after isolation and cannot be genetically modified. Recent research has demonstrated induced pluripotent stem cell-derived hepatocyte-like cells (iPSCdHLCs) as an appropriate model for HEV infection; capable of supporting HEV growth from all four genotypes and clinical isolates. However, PSC technologies have seldom been applied to relevant livestock species, specifically HEV's main zoonotic reservoir: pigs.

Here, we demonstrate porcine iPSCdHLC generation by comparing and adapting existing human differentiation protocols. Morphology and differentiation marker profiles(HNF4A, AFP, ALB) were compared between three different human PSCdHLC-adapted protocols. Our final protocol provided the most convincing hepatic phenotype and profile. Examination of differentiation markers by qPCR (Nanog, Sox2, Oct4, Gata4, HNF4A,AFP,ALB,GLUT2, HNF1B) showed improved hepatic expression profiles compared to previous porcine iPSCdHLCs and human PSCdHLC. The protocol generated here represents a tool for the study of HEV's zoonotic reservoir and we anticipate this system as a useful tool for host-pathogen interaction studies identifying porcine proviral and antiviral factors and for the development of HEV livestock vaccines.

A410

Genotype to phenotype consequences in MERS-CoV

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Abstract

Middle East respiratory syndrome coronavirus (MERS-CoV) has a reported mortality rate of 35%, making it the most pathogenic human coronavirus known. Dromedary camel populations are the major reservoir for MERS-CoV with spillover into humans causing sporadic infections. Human-to-human transmission has been confirmed, although only between close contacts in the same household or in healthcare settings. Currently, knowledge surrounding human-to-human transmission is limited and there are no specific medical countermeasures available to treat MERS-CoV disease. Therefore, it is necessary to improve understanding of the genotype to phenotype consequences in MERS-CoV that cause different pathogenesis, so that we can better respond to virus adaptation. To address this, mutations that occur naturally within the receptor binding domain and furin cleavage site of MERS-CoV spike during human infection were identified. A panel of MERS-CoV spike lentiviral pseudotypes containing these mutations were generated and expression of MERS-CoV spike protein, the firefly luciferase reporter and the lentiviral core genes were confirmed by Western blot. We show that the spike protein of the Dammam/19 MERS-CoV strain binds to human DPP4. Pseudotypes carrying this same spike sequence infect HEK293T cells expressing human DPP4 and were neutralised by pooled patient sera. Preliminary data also show that the mutations identified in clinical samples impacted infectivity and neutralisation. Identification of key residues that contribute to differences in MERS-CoV viral entry and immune evasion in humans will aid the development of therapeutics, while also improving our ability to respond to virus adaptation, which is of particular importance for pandemic preparedness.

A411

Epigenetic regulation of influenza viruses in diverse hosts

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Abstract

Influenza virus (IAV) frequently undergo mutation and provide threat to wide range of species. m6A modification is one of the most important post transcriptional modification, that possess various roles in virus replication and pathobiology. IAV is the first virus that has identified with m6A marks, however, its role in virus life cycle and host virus interactions is not yet explored. m6A modification and its impact has been explored in humans but not in other host species like chicken, pigs, horse and dogs which forms IAV transmission cycle. In order to understand impact of m6A marks in these species, we have focussed on m6A reader protein YTHDF2. P/Q/N rich N-terminal region includes processing bodies which degrade m6A modified RNA, recognised by m6A recognition region in C-terminal end, which also contains YTH domain. In this study we have cloned these three functional regions into piggy bac vector plasmid and its effect on virus replication kinetics has been studied. YTHDF2 was found to be cytoplasmic and its overexpression resulted in inhibition of virus replication. Stable cell lines expressing chicken and human YTHDF2; and KO cells using CRISPR-Cas9 has been developed for further experimental studies. Mutant viruses each containing single m^6A modification is employed to study IAV replication kinetics. Study on diverse host m6A machinery will aid to understand virus replication kinetics in different hosts involved in IAV transmission cycle. Insights into these influenza viral epitranscriptomics could explore several aspects in virus pathobiology, virus-host interactions which could lead to development of antivirals and vaccines

A412

Variant-dependent protective humoral immunity against SARS-CoV-2

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Abstract

The emergence of the novel immune-evasive variants of SARS-CoV-2 underlines the importance of vaccine adaptation. Analysis of immune response differences to evolving and disappearing variants is a key point of COVID-19 vaccine evolution.

The current research focuses on comparison of the pre-Omicron- and Omicron-induced humoral immune response indicators.

The sera of patients distributed into 2 groups: pre-Omicron (n = 119) and Omicron (n = 158) etiology) were examined for anti-RBD IgG, intensity of humoral immune response and IgG avidity by ELISA for 45 days after the onset of disease symptoms. The pre-Omicron (n = 30) and Omicron (n =20) IgG cross-reactivity was estimated in live virus neutralization assay with Delta and Omicron variants using Vero E6 cell line. Statistical analysis: Mann-Whitney U test, Chi-squared test, Wald method.

The significant difference in kinetics of seroconversion (up to 12 days, $p < 0.001$) and IgG concentration levels (throughout the entire period, $p < 0.005$) to RBD SARS-CoV-2 was observed in 2 groups of patients with absolute predominance of pre-Omicron-associated immune response. Despite the less intense Omicron-induced immune response, it was characterized by more pronounced production of high-avidity antibodies (36,7% [23,4%52,9%] in comparison to 8,1% [1,2%;26,3%] with pre-Omicron-caused response).

The IgG cross-reactivity studies revealed that the median titers of anti-Omicron IgG neutralizing Delta and Omicron viruses were 1:32 and 1:16, respectively. The reversed test of pre-Omicron-induced IgG neutralizing ability showed median titer 1:64 against Delta virus and 1:8 against Omicron variant with registered indicator below this value in 66.7% [48.8%;80.9%] of the examined individuals.

A414

Comparing antibody function in acute and chronic Hepatitis C virus infected patients against autologous and heterologous virus glycoprotein clones.

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Abstract

Hepatitis C virus (HCV) infections represent a global public health burden, and the development of a prophylactic vaccine remains essential. Despite the availability of direct-acting antiviral drugs, the elimination of the virus remains challenging. Around 30% of infected people can clear the virus (acute infection), whilst the other 70% become chronically infected. The exact mechanisms behind this are not yet known. The humoral immune response has been the focus of vaccine development in recent years, but concentrates on the neutralising ability of the antibodies, not their ability to engage other aspects of the immune system. Here we investigated whether there were differences in the antibodies elicited by acute or chronic HCV infection. This was done by studying antibody binding, pseudotype neutralisation, and antibody-dependent cellular cytotoxicity (ADCC) with NK cells to both autologous and heterologous HCV E1E2 glycoprotein clones. Acute-infected patients exhibited both greater binding and neutralisation of an autologous E1E2 clone, compared to a heterologous clone. Chronically infected patients however, exhibited a wider breadth of binding and neutralisation. Antibodies from acute-infected patients were also able to elicit NK mediated ADCC more efficiently than chronically infected. This study provides initial evidence that ADCC responses could be important in HCV infections and can provide useful insights for future studies on therapeutics and potential vaccine candidates.

A415

Decoding differential activity of native and recombinant type I interferons against Rift Valley fever virus infection

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Abstract

Interferons (IFNs) form a crosstalk between innate and adaptive immunity, dictate the strategy of the initial and long-term immune response and subsequently have drastic impacts on viral life cycle. IFNs have been extensively used for clinical management of selected viral infections, but their use could be extended to first line treatment during outbreaks of emerging viruses. Rift Valley fever virus (RVFV) is a World Health Organization (WHO) priority pathogen with the potential to spread outside of the endemic areas and currently no specific approved treatments. Here we show a comparative analysis of native human type I IFNs and recombinant IFNs in their effectivity as pre- and post-prophylaxis treatments against RVFV in *in-vitro* models We performed viral inhibition assays and transcriptomic analyses which revealed that IFNs have a differing spectrum of antiviral activity against RVFV, qualitative range of downstream products, and temporal expression levels of different interferon stimulated genes. Altogether our data displays that rational design of IFN sequences can lead to their differential antiviral activity, cytokine induction profile and increase of potency independent of dosage. A better understanding of what underlies the functional differences of IFN subtypes presents an promising opportunity for targeted IFN therapies with limited side effects.

A416

Worms go viral: The diversity of RNA viruses in parasitic nematodes and their interaction with host immunity.

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Abstract

From screening published transcriptome data, we have identified 85 different virus genomes and virus-like sequences within 28 species of parasitic nematodes. As these parasites infect >1.5 billion people and animals globally, this finding may have broad-ranging impacts for almost a quarter of humanity and economically important livestock. Our analysis shows extensive diversity and a conserved global spread of virus/nematode associations across multiple continents suggesting an ancestral acquisition event and host-virus co-evolution. Focussing on the viruses of the filarial parasites *Brugia malayi* (Togaviridae, related to Alphaviruses) and *Onchocerca volvulus* (Rhabdoviridae, related to Lyssaviruses) reveals an intimate relationship, with the viruses localising within the reproductive tracts of both species, and being detectable in all laboratory isolates (*B. malayi*) as well as multiple isolates across Sub-Saharan Africa (*O. volvulus*). Viruses of *B. malayi* were also found to localise within cuticular inflations, or 'bosses' that were previously described in the literature, but with no known purpose. Additionally, we were able to show that the final mammalian host of these filarial parasites elicit antibody responses against the viruses demonstrating exposure to host immunity. This observation, as well as the intimate, chronic and life-long exposure to vertebrate tissues that typically characterise parasitic nematode infections, raise questions as to the role this previously hidden virome plays in modulating anti-parasite immunity, and their potential for driving disease pathogenesis, such as onchocerciasis-associated epilepsy.

A417

Phenotypic characterization of a mycovirus infecting an insect pathogenic fungus

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Abstract

Beauveria bassiana is an entomopathogenic fungus that has been established as an important pest control tool in agriculture. The recent advent of the Polymycoviridae family has been of particular interest due to its ability to increase the pathogenicity of B. bassiana against insects such as the greater wax moth Galleria mellonella and the army mealworm Tenebrio molitor. Our study on Beauveria bassiana polymycovirus 1 (BbPmV1) aims to characterize the specific phenotypic advantages and disadvantages that BbPmV1 confers on its host under different conditions. Through radial growth and metabolic assays using B. bassiana strain EABb 92/11-Dm, we have established significant differences between the virus-free and BbPmV1-infected isogenic lines. Our results suggest that BbPmV1 influences its host growth rate and metabolic activity on media with different carbon and nitrogen sources and under a wide range of conditions including temperature and osmotic stress. These findings lay the foundations for a better understanding of a symbiotic relationship between polymycoviruses and their hosts and will guide the molecular analysis of the underpinning mechanisms in the future.

A418

Coronavirus poly(A) tail length regulation by host polyadenylation machinery

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Abstract

Coronaviruses have a positive-sense single-stranded RNA genome, with a conserved hexamer in the 3′ untranslated region that resembles a host eukaryotic poly(A) cleavage signal. While the viral RNA remains in the cytoplasm during infection, variation of its poly(A) tail length has been reported, suggesting potential involvement of host polyadenylation machinery in the generation of the coronavirus poly(A) tail. Contrary to previous reports, we observed a viral poly(A) tail length at ~60 nt for SARS-CoV-2 and ~45 nt for HCoV-OC43 with minimal variation. The length of viral poly(A) tail is captured and compared at early timepoints post high MOI infection by 1) ligating a DNA linker to the 3′ end of vRNA, followed by reverse transcription and PCR; and 2) direct RNA sequencing. To investigate the roles of host polyadenylation complex during infection, we have established cell lines with an auxin-inducible degron system, which allows fast degradation of essential host proteins. As the poly(A) tail is a common feature in multiple positive-sense RNA virus families, its regulation may shed light in the viral replication cycle in general.

B001

In-situ automated microbial imaging

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Abstract

Measurements of Optical Density (OD) are the standard way of estimating the growth curves of micro-organisms. In a given range of lower microbe densities, the OD measurements are proportional to cell numbers. However, the proportionality no longer holds for applications where there are changes in cellular size or refractive index (RI), or if the growth media is turbid (e.g. contains particles that scatter light) or changes the RI. Furthermore, even without those changes, the OD measurements at higher culture densities become inaccurate due to 'multiple scattering'. In those cases, OD cannot be used to estimate growth efficiently. One solution to this problem is to directly image these cells because live cell imaging allows tracking of changes in cell morphology and number in real time. Morphology holds information on the underlying molecular processes, however is not easy to access with high throughput. Therefore the information is not accessible and currently exploited. Single-cell studies on bacterial species are particularly difficult to carry out due to their size and their ability to 'swim'. Here we show, that single-cell imaging of a growing cell culture, achieved by imaging in a 2D plane in the vessel the cells are growing in, allows for the observation of cell number and physiology in high quality, as well as allows to acquire a large set of imaging data. We demonstrate how data can be used to perform a direct cell count over time from the device, as well as to observe the cells over their respective life cycles.

B003

Surveillance of extended-spectrum beta-lactamase-producing Enterobacterales in care settings across Liverpool

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Abstract

Infections with extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) pose an increasing clinical threat. Drug resistance conferred by these bacteria reduces treatment options and increases healthcare costs. The absence of effective treatment can make healthcare-associated infections life-threatening. Risk factors for acquisition of ESBL-E carriage, a key step preceding infection, are not well defined in the UK, though those with high care needs and/or living in long-term care facilities are thought to be at increased risk.

Hence, we have established a surveillance platform in Liverpool for the detection of ESBL-E in healthcare settings across the care spectrum: acute hospital wards (n=4), intermediate care facilities (n=1) and care homes (n=2). Our sampling strategy involves collecting repeated rectal swabs or stool samples from patients, alongside sampling the surrounding healthcare environment. We then use culture and molecular methods to identify ESBL-producing *E. coli* and *K. pneumoniae* from these samples.

As of December 2023, we have collected 321 samples from 94 patients and confirmed the presence of ESBL-producing *E. coli* or *K. pneumoniae* in 26 (27.7%). In the environment, 6.5% of 1825 swabbed locations were ESBL-positive. Most ESBL-E isolates came from hand wash sinks (55.8% of all ESBL-positive samples), followed by shower drains (29.2%) and toilets (15.0%). No high-touch surfaces (nurses' stations, door handles, computer keyboards) were ESBL-positive.

These findings, along with genomic data to follow, will be used to infer risk factors for ESBL-E acquisition. Ultimately, this data will inform the design of interventions to prevent acquisition and transmission of ESBL-E in healthcare systems.

B004

Does routine microbial contamination of powdered infant formula milk pose an unrecognized food safety risk for infants?

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Abstract

Powdered infant formula milk (PIF) is not sterile, therefore, preparation advice for parents is strict. However, recent work has shown advice is not highly adhered to. This study investigates the microbial contamination within PIF across brands and product types. It looks at this in combination with different at-home preparation techniques and the occurrence of antimicrobial resistant microorganisms. PIF products were prepared in parallel with a popular at-home preparation machine (AHPM), 70°C (NHS guidelines), or room temperature sterile water and plated onto tryptic soy (TSA) and Sabouraud agars (SAB). Isolates were identified using MALDI-ToF mass spectrometry. For AMR microbe isolation, PIF was incubated with buffered peptone water and dilutions of 11 clinically relevant antibiotics belonging to 7 classes (128 mg/L to 1 mg/L). This was subcultured onto TSA, milk agar, agar enriched with 2% PIF or SAB agar. These were cultured aerobically at 30°C, 37°C or at 8% CO_2 at 37°C. All preparation methods resulted in microbial growth with no significant difference observed between room temperature and 70°C preparation ($P<0.05$). AHPM resulted in significantly more contamination of PIF products. Isolates include opportunistic pathogens associated with infant illness e.g. *Bacillus cereus* and *Cupriavidus pauculus*. *Bacillus spp*. were isolated showing acquired resistance to erythromycin, vancomycin and imipenem. Current PIF preparation methods are insufficient at eliminating the risk of bacterial contamination and commonly used AHPMs may increase microbial contamination. The impact of routine microbial contamination of PIF on infant health is currently unknown and must be investigated further.

B005

Concurrent study of poultry enterotypes and resistomes may enable the detection of acquired antimicrobial resistance

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Abstract

The poultry industry in Asia is at high risk for the emergence of antimicrobial resistance (AMR) because of the widespread use of antibiotics. Poultry are also natural hosts of zoonotic pathogens such as *Campylobacter*, *Salmonella* and *Escherichia coli,* capable of causing severe illness in humans. Identifying poultry farming practices that decrease the abundance of zoonotic pathogens and antimicrobial resistance genes (ARGs) is needed to lower the potential for the emergence of acquired resistance in these disease-causing species. However, surveillance of AMR is notoriously difficult because i) ARGs can be spread between species via horizontal gene transfer, and ii) the vast majority of ARGs in any resistome represent the normal innate resistance of a microbial population- making the detection of an acquired AMR signal very difficult to detect against the innate AMR background.

Enterotypes represent distinct microbial community phenotypes and resistomes represent population AMR phenotypes. Variations in both can have significant effects on chicken health and public health risk. Using 16S rRNA amplicon sequencing to define community composition and AMR AmpliSeq for ARG composition- we identified a striking correlation between enterotype and resistome. This correlation between enterotype and resistome likely reflects that the majority of ARGs in a resistome represent the innate AMR of the species of which the population is composed. Interestingly, we also identified sample outliers- where the enterotype-resistome correlation was weak. We explore the hypothesis that these outliers may represent samples in which resistance has been acquired.

B006

Resistance to empirical antibiotics used in urinary tract infections at the Norfolk and Norwich University Hospital.

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Abstract

The rise of antimicrobial resistance in urinary tract pathogens poses a challenge to clinicians managing these infections. We analysed antibiotic resistance in urine samples sent to the Norfolk and Norwich University hospital between 2018 and 2023. Samples from non-pregnant adults with gram negative bacilli in the urine were included. Over the five-year period, 73,318 samples met inclusion criteria. Antibiotic sensitivities were determined by disc diffusion.

The most common organism was *E. coli*, isolated in 70% of cases, followed by coliforms in 14% of samples. Trimethoprim resistance was high; detected in 25.4% of isolates. Cefalexin and nitrofurantoin resistance was lower at 8.6% and 9% respectively.

We analysed 126 isolates with cefpodoxime resistance on disc diffusion. Sensitivities to additional agents including pivmecillinam and fosfomycin were available for these samples. The most abundant organisms were *E. coli* (90 isolates) and *Klebsiella pneumonia* (14 isolates). 47 isolates were positive for AmpC and 75 were ESBL producers. Sensitivity to pivmecillinam was detected in 88% of isolates via disc diffusion. Fosfomycin sensitivity was detected in 80%; 20 were sensitive via disc and 69 had an MIC <16, determined by the VITEK 2 XL system.

Our research depicts high levels of resistance to trimethoprim but moderate resistance to other antimicrobials. Of concern is that trimethoprim is a first line agent for the treatment of urinary tract infections within our trust. Future work will include revision of guidelines to incorporate local resistance patterns and to elucidate the prevalence of AmpC and ESBL isolates within our area.

B007

Surveillance and phenotypic characterization of antimicrobial resistance profiles in complex rumen microbiome samples.

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Abstract

As one of the most serious threats to humans, antimicrobial resistance (AMR) can spread between humans, animals and the environment, contributing a poor implication on One Health. Recent research shows that culturable rumen bacteria harbor antibiotic resistance genes (ARGs) with the potential for horizontal gene transfer. However, many bacteria in the rumen microbiome are unculturable, limiting our understanding of the extent of transmissible ARGs and ARG reservoirs present in this ecosystem. Given the enormous One Health implications of this, and to advance our understanding of AMR risk in this ecosystem, we attempted to improve the culturability of as yet uncultured anaerobic rumen bacteria and subsequently characterize their phenotypic and genotypic antimicrobial resistance profiles. Using the dilution to extinction culturomics approach encompassing 3 media types (Hobson M2 media, Brian Heart Infusion media and PC basal media), we successfully isolated 84 isolates from 3 ruminal fluid samples from bovine/sheep, one of which was a novel gram-negative anaerobic *bacillus* identified by 16S rRNA sequencing with just 97.2% identity to *Massilibacteroides vaginae* strain MV12. Furthermore, we also show evidence of the presence of isolates not previously reported in the rumen including *Winkia neuii subsp. Anitrata, Virgibacillus pantothenticus*, and *Caldibacillus hisashii,* which also failed to be identified by MALDI-TOF. We are currently assessing the AMR phenotype of this new isolate and performing whole genome sequencing to analyze the potential expressiveness and mobility of the AGRs in this rumen isolate which will provide further insight into the resistome in this new isolate.

B008

Characterising microbial communities with metagenomic De Bruijn graphs

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Abstract

In the face of the evolving challenges posed by global pandemics like COVID-19 and the growing impact of climate change, advanced analytical techniques for microbial surveillance are essential. This study explores a novel approach using coloured De Bruijn Graphs (cDBGs) for environmental metagenomic surveillance, offering a departure from conventional Operational Taxonomic Unit (OTU) analysis. cDBGs integrate temporal sequence-level data into a linear branching structure, with colours representing sample collection times in longitudinal studies. This approach holds the potential to provide deeper insights into microbial community dynamics and simplify the interpretation of complex metagenomic datasets.

Preliminary results, based on the analysis of publicly available longitudinal wastewater metagenomics datasets, reveal promising insights into the use of graphs to understand microbial diversity and community evolution over time. However, this application of cDBGs in metagenomics is still in its nascent stages, necessitating further validation and research to establish meaningful connections between graph properties and biological signals.

The utilisation of cDBGs in metagenomic analysis offers significant potential, particularly in deciphering the complexities of dense microbial datasets. This approach may lay the foundation for characterising a baseline for environmental metagenomes and detecting emerging pathogens in the environment. Future research will focus on refining and validating this approach, paving the way for enhanced microbial surveillance and a deeper understanding of microbial populations in complex samples.

B009

Optimising an end to end protocol for clinical metagenomics on respiratory samples

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Abstract

Clinical metagenomics has great potential for infection diagnosis, surveillance and research. These benefits are significant in respiratory infections where standard clinical microbiology methods frequently yield no results. In addition, the emergence of novel respiratory viruses such as SARS-CoV-2 has highlighted the importance of unbiased surveillance. This study seeks to optimise protocols for sample collection, nucleic acid extraction, sequencing, bioinformatic analysis and clinical reporting.

Respiratory samples were collected from patients admitted to the acute medical and Intensive care wards at the Queen Elizabeth Hospital Birmingham. Samples were collected based on clinical availability, a nasopharyngeal swab and where ever possible sputum and additional respiratory samples were received. Control samples were obtained from individuals not thought to have infection. A case report form was completed for each patient providing comprehensive metadata.

Where necessary, samples underwent a human cell depletion step, before total nucleic acid extraction on a ThermoFisher kingfisher instrument. cDNA was synthesised and the whole sample prepared for sequencing on the ONT gridION. Commercial, published and in house bioinformatic pipelines were validated for results analysis. A set of control organisms representative of those expected to be found in the respiratory tract were used alongside commercially available controls to ensure quality control and a provide a measure of sensitivity of the assay. Various metagenomic phylogenetic analyses were employed to identify the presence of any microbial pathogen within the samples.

This study demonstrates the importance of method optimisation and validation for clinical metagenomics to reach its full potential.

B010

A Novel Wet Lab Protocol for Amplicon Concatenation Targeted for Long-Read Sequencing

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Abstract

Amplicon sequencing is a cost-effective and widely used method for investigating the taxonomic diversity of microbial communities from a vast range of complex matrices. Illumina sequencing has been the commonly implemented method to investigate microbial community diversity by targeting a small number of short hypervariable regions of shared genetic homology. However, the inherent limitation of sequence length associated with Illumina platforms restricts taxonomic identification. These limitations can be circumvented by using long read sequencing platforms which can provide a more comprehensive view of microbial diversity in complex samples. The comparatively higher cost and lower throughput of long read sequencing platforms relative to Illumina platforms is therefore the current limitation in taxonomic resolution using amplicon sequencing to profile microbial communities.

Our novel protocol amplifies full length taxonomically relevant genes (e.g. full length 16S and ITS regions) from diverse matrices then subsequently concatenate and barcode these products into a linear construct via a simple and controllable "one pot" polymer assembly reaction. This provides a template for high throughput long read sequencing platforms which is tuneable in terms of concatemer length, gene order and orientation. Background DNA and constructs that have failed to fully assemble are then removed via a novel clean-up step within the one pot protocol. Here we present data from a range of complex matrices which include mock communities, faeces, and soil.

B011

Clinical metagenomics for viral diagnostics using short-read, long-read and targeted approaches

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Abstract

Metagenomics, the sequencing of all genomic material within a sample, is a demonstrably powerful approach for detection of novel or unknown pathogens. Workflows based on Illumina short-read sequencing are now well established in some diagnostic laboratories. Technologies such as Oxford Nanopore sequencing could allow analysis of data in real time, providing the possibility of point-of-care testing. Targeted approaches could also be used to improve sensitivity but are currently not widely used in diagnostics.

We performed a comprehensive evaluation of easily available untargeted Illumina and Nanopore metagenomics sequencing workflows and an Illumina-based capture approach using the Twist Biosciences Viral Research Panel for viral metagenomics. We also compared the performance of commonly used bioinformatics methods. Initially, we tested mock samples with known viral loads, designed to resemble clinical specimens with a high host DNA/RNA content. We then applied the protocols to residual clinical samples where pathogens have been identified by other methods.

Nanopore-based metagenomics provides similar sensitivity and specificity for viral detection compared to Illumina at high viral loads, so may be the optimal platform where fast turnaround times are required. At low viral loads, higher sequencing depths with longer turnaround times compared to Illumina may be required to increase the sensitivity of Nanopore-based sequencing. Targeted approaches provided increased sensitivity to viruses but may reduce the ability to detect untargeted organisms. Use of robust thresholds for the classifiers helps standardise performance of bioinformatics pipelines in terms of both sensitivity and specificity.

B012

Oxford Nanopore Technologies 16S sequencing in routine clinical practice: implementation, impact and further directions

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Abstract

Rapid identification of microbiological organisms is especially important in today's healthcare setting as we face increased antimicrobial resistance. Diagnostically there is a clear need for a rapid, accurate and flexible bacterial identification assay that could be run in a routine diagnostic laboratory, while remaining cost effective and lean.

The 16S rRNA gene codes for the 16S ribosomal RNA component present within all prokaryotic ribosomes. Sequencing of the 16S rRNA gene has been widely accepted as a supplementary investigation in clinical microbiology laboratories since the principle was first described in 2003. The current gold standard for sequencing the 16S gene in clinical diagnostic laboratories is a Sanger sequencing method. In a national first, Liverpool Clinical Laboratories (LCL) has introduced a routine Oxford Nanopore Technologies (ONT) sequencing method for the full 16S gene in May 2023. During our evaluation we have clearly demonstrated increased sensitivity and specificity compared to the gold standard method. We have since evaluated the laboratory and clinical impact of this diagnostic test, focusing on the useability of the assay; the impact on patient outcome and when and why in the patient journey the assay is being ordered with a view to developing national guidelines and introducing genomics into routine NHS services.

We are the first centre to make 16S ONT sequencing part of our routine sequencing repertoire and the optimised ONT 16S workflow successfully circumvents the documented limitations of the current Sanger method and is now an invaluable part of our routine diagnostic repertoire.

B013

Quantitative microbial risk analysis of antimicrobial resistant environmental *Escherichia coli* from irrigation water

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Abstract

While antimicrobials as growth promoters are banned in the majority of the countries, their therapeutic use in livestock still contributes to the generation and environmental spread of antimicrobial resistance (AMR). As a key aspect of the One Health approach, the identification of AMR spread between and among humans, animals and the environment is pivotal, and it could lead to a decreased risk posed by the harmful bacterial and AMR persistence in the ecosystem and therefore a decreased risk of exposure to harmful bacterial and AMR. The transmission of *Escherichia coli* to the environment via irrigation water represents a quantifiable risk to the food chain. Moreover, the quantification of the risk to health from low infection doses could contribute to assessing the relationship between bacterial concentrations and the development of infection, as some *E. coli* strains could be present at the low number in the environment.

This project aims to develop a quantitative risk assessment of *E. coli* and the relative AMR hazard via developing a quantitative microbial risk analysis (QMRA). We carried out an *in vitro* experiment to generate *E. coli* survival data in irrigation water, used to inform exposure assessment, one of the essential steps of QMRA development. The dose-response model is based on Monte Carlo simulations, whose output depicts the probability of infection due to exposure to environmental *E. coli*. Furthermore, the QMRA generates likelihood figures characterising the risk to AMR exposure based on the quantitative analysis of an intrinsic resistance gene (*tet34*) from the *in vitro* experimentation.

B014

Metagenomic insights into plant-based foods: Unveiling diversity across different protein sources

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Abstract

The increasing demand for sustainable and nutritious meat alternatives has led to the development of a diverse range of plant-based meat alternative products utilising different protein sources, including soya, pea, wheat, and mycoprotein.With the emergence of these products on the market, research is delving into the associated microbial communities,exploring their relationships with other parameters including, the product characteristics and the influence of the manufacturing process on their safety and quality.

In this study, we used culture-dependent and independent methods, Sanger sequencing and Illumina MiSeq 16S and ITS metagenomic sequencing, to characterise the bacterial and fungal microbiota of (total of 38) plant-based meat-alternative products from across the European market, from various manufacturers, utilizing different protein sources. Products were analysed at expiry date and 7 days post-expiry. The richness and evenness of the sample were analysed through alpha and beta diversity. The influence of factors including protein source, manufacturer, pH, water activity, moisture, salt, and preservatives on the diversity of microbial communities was evaluated.

Plant-based foods seem to exhibit a unique microbial profile characterized by a rich diversity in microbial communities. Lactic acid bacteria - *Leuconostoc, Agrilactobacillus* and *Latilactobacillus* were detected as spoilage organisms in products with high water activity.Anaerobic bacteria like *Anaerocolumna* were primarily found in mycoprotein- based food along with *Sphingomonas* which was abundant across most of the products.*Candida* and *Cyberlindnera* are salt-tolerant and have osmotic adaptation ability, predominantly found in high-salt-concentration food.Spore formers like *Clostridium* and *Aneuribacillus* were found in soya and wheat-based food. Bifidobacterium,*Curtobacterium, Weisella, Asticcacaulis, Termus, Aneuribacillus , Methylobacterium, Kocuria, Lawsonella, Paracoccus,* and *Clostridium* were found across food products.

B015

Safe Waste - A surveillance study of viruses and parasites present in organic waste used as fertiliser in Ireland.

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Abstract

In Ireland animal faeces, urine, and bedding generated on farms are spread on land as organic fertilisers to promote the growth of grass and certain commercial crops. Despite this practice being commonplace, its safety in Ireland has yet to be characterised from a microbial standpoint, particularly with regards to parasites and viruses.

There is a growing movement to reduce the use of chemical fertilisers and to reduce organic waste entering landfills, thus resulting in the land spreading of organic waste. Therefore, there is an urgent need to characterise this waste and identify pathogens which may pose a risk to public health. This assessment of the viral and parasitic safety of organic waste in Ireland is part of a wider project entitled Safe Waste. This project aims to characterise and mitigate both chemical and microbial contaminants in organic waste in Ireland.

This presentation will detail findings to date on the prevalence of the parasite Cryptosporidium parvum in multiple organic waste streams and the prevalence of the birnavirus Infectious Bursal Disease Virus (IBDV) in chicken litter. While C. parvum is a zoonotic pathogen and poses a direct risk to public health, IBDV is seen to have an indirect effect. By dampening the immune response of poultry, IBDV leaves infected birds susceptible to other zoonotic pathogens such as avian influenza and Campylobacter spp., which in turn have the potential to pose a risk to public health if contaminated litter is spread on land. In addition to the risk to public health, it is also essential to mitigate the spread of pathogens through the environment to protect animal health in Ireland.

B017

PCR cycle optimisation: Implications for metagenomic next-generation sequencing (mNGS) in low biomass samples

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Abstract

Introduction

Metagenomic Next-Generation Sequencing (mNGS) offers a cultivation-free method for studying microbial communities. However, the challenge lies in obtaining sufficient nucleic acid, particularly for low-biomass samples. Additional PCR amplification during library preparation enhances nucleic acid concentration but introduces potential composition bias. Our goal is to quantify the impact of varying PCR cycles on mNGS samples.

Material & Methods

We exposed 40 nasal swabs to four distinct conditions: undiluted with 6 PCR cycles, and a 1:10 dilution at PCR cycles 6, 9, and 12. Subsequently, the samples underwent shotgun metagenomics using Illumina MiSeq (300bp paired-end, multiplexed with 96 samples).

Results

A higher PCR cycle resulted in an increased read count, and the rise in DNA concentration significantly correlated with the heightened read count ($p<0.01$). Alpha diversities showed no significant differences between PCR cycles and DNA concentrations. While intrasample beta diversity exhibited significance ($r^2 = 0.00004$, $p<0.05$), intersample diversity did not, indicating that PCR cycles introduce some bias but not enough to impact overall taxonomy composition.

Applying PhILR, we noted a substantial shift in phylum balance among samples. There was a significant enrichment of Actinomycetota reads ($p<0.01$) and a depletion of Bacteroidetes reads ($p<0.01$) with increasing PCR cycles. The investigation into the quality of Metagenome-assembled genomes subjected to different PCR cycles is currently underway.

B018

The Detection of *qnrA, qnrB* and *qnrS* Genes in Soil Bacteria Using Loop-mediated Isothermal Amplification (LAMP)

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Abstract

Background

The Quinolone class of antibiotics are used to treat bacterial infections in human and veterinary medicine.

As with other classes of antibiotics, resistance to quinolones has been reported. Development of plasmid mediated resistance is of particular concern as it allows for the horizontal spread of quinolone resistance genes, such as *qnrA, qnrB* and *qnrS,* between bacteria.

Soils support a complex ecosystem of micro-organisms. Human activity, such as farming or improper/overuse of antimicrobials, contaminates soils with antibiotics and forces an evolutionary shift in bacteria towards developing antibiotic resistance. Detection of antibiotic resistance genes is vital.

Loop-mediated Isothermal Amplification is an isothermal, single tube technique used for DNA amplification and gene detection with a simple yes or no result. Compared to PCR, LAMP has been demonstrated to be up to 10 times more sensitive, as well as being more tolerant to contaminants found in soil samples that can typically hinder PCR.

Methods

Soil samples were inoculated with *E. coli* containing *qnrA* and *qnrB* genes and *K. pneumoniae* (NCTC 13439) with the *qnrS* gene and then DNA extracted. LAMP assay protocols were developed and evaluated to detect *qnrA, qnrB* and *qnrS* genes.

Results/Discussion

The LAMP assay was able to detect the *qnr* genes with the detection of the *qnrA* gene being far more pronounced than *qnrB*. This possibly could be improved with further optimization of the LAMP primers and protocols. The evaluation of the *qnrS* LAMP assay is ongoing.

Conclusion

LAMP could play a pivotal role in the detection and monitoring of antibiotic resistance genes in soil.

B019

Evaluating the faecal microbiota and metabolome of dogs affected by Canine Cutaneous and Renal Glomerular Vasculopathy using 16S rRNA gene profiling and ^{1}H Nuclear Magnetic Resonance

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Abstract

Canine Cutaneous and Renal Glomerular Vasculopathy (CRGV) is a frequently fatal, idiopathic disease characterised by cutaneous lesions and vasculitis of the glomeruli, often resulting in acute kidney injury. The aetiology of CRGV remains unknown, although a bacterial cause has been hypothesised. In this study, the faecal microbiota and metabolome were examined in healthy (***n***=96) and CRGV-affected dogs (***n***=104) using 16S rRNA sequencing and ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy, respectively, testing the hypothesis that CRGV is associated with distinct changes in the bacterial gut microbiota and associated metabolome. Faecal microbiota profiling was conducted following DNA extraction, amplification of the V4-V5 regions of the 16S rRNA gene, and sequencing of the subsequent amplicons. Amplicon Sequence Variants (ASVs) were produced using exact sequence similarity via QIIME2 using DADA2. Metabolomic profiling was carried out on faecal water using an NMR spectrometer, and the spectral profiles were interrogated using multivariate statistical analysis tools (OPLS-DA) in MatLab. Alpha diversity (Shannon entropy/total observed ASVs) showed no significance between healthy and CRGV-affected dogs (***p***>0.05). The taxonomic composition of the CRGV-affected dogs revealed increases in the bacterial families Enterobacteriaceae and Enterococcaceae during disease. This study also identified metabolites linked to bacterial metabolism that significantly differed between the CRGV-affected and healthy dogs, including choline, valine, alanine, and propionic acid, respectively. The findings may offer a potential explanation for the clinical signs observed upon presentation, such as lameness, lethargy, and anorexia. This is a novel study being the first to characterise the faecal microbiota and metabolome of CRGV-affected dogs.

B020

Development and validation of a PCR and next generation sequencing assay to diagnose viral infection from faecal samples.

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Abstract

Diagnosis of symptomatically identical diseases can be laborious and expensive as multiple tests would be required to distinguish which infectious agent is responsible for causing disease. Additionally, the chance of misdiagnosis is increased should an animal be concurrently infected with two viruses, which most commercial tests do not cover. This challenge is amplified in intense farming situations where it's not practical or financially feasible to sample all animals on farm. This study aimed to create a universal diagnostic test that could detect low copy numbers of virus in a faecal sample, allowing for non-invasive bulk herd or flock sampling. The focus was on diagnosis of single strand RNA avian respiratory virus influenzas A virus (IAV) and Newcastle disease virus (NDV). To optimise the test a chicken faecal sample was spiked with IAV and/or a vaccine strain of NDV, we then trialled RNA treatments to reduce the proportion of bacterial RNA present, then using sequence independent single primer amplification (SISPA) amplified each sample to a quantity of nucleic acids required for oxford nanopore sequencing. The test was able to detect as low as 10^3 copies of virus. The test was validated in an outbreak scenario.

B021

Development of a Field-Deployable Isothermal Assays for Sensitive and Rapid Detection of Arboviruses in the Philippines

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Abstract

Mosquito-borne arboviruses, including Dengue (DENV), Zika (ZIKV), Japanese Encephalitis (JEV), and Chikungunya (CHIKV), pose a significant public health burden in the Philippines due to their widespread presence and overlapping geographic distribution. These viruses often cause similar symptoms, making clinical diagnosis challenging. While DENV, JEV and CHIKV continue to be one of the most important human diseases caused by arboviruses, recent years have seen a resurgence of ZIKV, highlighting the need for rapid and sensitive diagnostic tools.

Currently available methods, such as RT-PCR and serology, require specialized laboratories and are often inaccessible in remote areas where outbreaks occur. This delay in diagnosis hinders timely patient management.

To address these challenges, field-deployable isothermal assays for arbovirus detection were developed. This current study was able to develop assays employing the quencing of unincorporated amplification signal reporters in reverse transcription loop-mediated isothermal amplification (QUASR-RTLAMP) to simultaneously detect the viruses.

The developed QUASR-RTLAMP simultaneously detect the four arboviruses. On the basis of detection by fluorescent signal, where a positive for RNA fluoresce, it provides a simultaneous yes or no answer for the presence of arboviruses upon light excitation. We found that all assays perform optimally at 60°C achieving detectable amplification in 60 minutes. Our results also showed that DENV, JEV, ZIKV, and CHIKV have low detection limits, without cross-reactivity.

By providing a rapid and accessible diagnostic tool for arbovirus detection in the Philippines, this research will significantly improve outbreak response and disease control efforts, ultimately improving public health in the country.

BLOCK B

Session : Genetics and Genomics Forum

B022

Fleas of fleas: The potential role of bacteriophages in *Salmonella* diversity and pathogenicity

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Abstract

Non-typhoidal salmonellosis is an important foodborne and zoonotic infection that causes significant global public health concern. Diverse serovars are multidrug-resistant and encode several virulence indicators, however, little is known on the role prophages play in driving these characteristics. Here, we extracted prophages from 75 *Salmonella* genomes, which represent the 15 most important serovars in the United Kingdom. We analysed the genomes of the intact prophages for the presence of virulence factors which are associated with; diversity, evolution and pathogenicity of *Salmonella* and to establish their genomic relationships. We identified 615 prophage elements from the *Salmonella* genomes from which 195 prophages are intact, 332 being incomplete while 88 are questionable. The average prophage carriage was found to be more prevalent in *S*. Heidelberg, *S.* Inverness and *S.* Newport (10.2-11.6 prophages/strain) compared to *S*. Infantis, *S.* Stanley, *S*. Typhimurium and *S*. Virchow (8.2-9.0 prophages/strain), *S*. Agona, *S.* Braenderup, *S.* Bovismorbificans, *S.* Choleraesuis, *S.* Dublin, and *S.* Java (6.0-7.8 prophages/strain), and *S*. Javiana and *S.* Enteritidis (5.8 prophages/strain). Cumulatively, 2760 virulence factors were detected from the intact prophages and associated with cellular functionality linked to effector delivery/secretion system (73%), adherence (22%), magnesium uptake (2.7%), resistance to antimicrobial peptides (0.94%), stress/survival (0.4%), exotoxins (0.32%) and antivirulence (0.18%). Close and distant clusters were formed among the prophage genomes suggesting different lineages and associations with bacteriophages of other *Enterobacteriaceae*. We show that diverse repertoire of *Salmonella* prophages are associated with numerous virulence factors, and may contribute to diversity, pathogenicity and success of specific serovars.

B023

Measuring drivers of plasmid transfer

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Abstract

Horizontal gene transfer (HGT) can promote the spread of antimicrobial resistance (AMR) genes between bacteria, with conjugation being a major mechanism for the transfer of AMR genes by conjugative mobile genetic elements (MGEs), including plasmids. The spread of drug resistance in bacteria has led to a global AMR crisis, with the rise in the number of AMR infections of great concern.

Many infections involve a biofilm component, and these infections are often chronic due to the intrinsic AMR properties of biofilms. This can lead to increased, prolonged use of antibiotic treatment. In addition to their AMR properties, the proximity of cells within a biofilm makes them excellent hotspots for HGT. Although it has been established that high-level resistance can evolve through HGT within biofilms, the factors that impact the transfer of MGEs and evolution of resistance in these communities remain unclear.

To study this, we established a model multispecies biofilm community using *Escherichia coli* and *Salmonella enterica* serovar Typhimurium and screened a range of plasmids for genes that confer resistance to clinically important antibiotics using a variety of bioinformatic tools. These plasmids were characterised in terms of their mobility and suitability for use in the model multispecies biofilm community. We describe here the characterisation and evolutionary history of a unique IncFII plasmid carrying *bla*$_{CTX-M-14}$ and how we optimised the model to monitor the impact of different environmental stressors on the movement of this plasmid within multispecies biofilm communities.

B024

Outbreak of XDR typhoid in Punjab Province of Pakistan

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Abstract

Introduction: Extensively drug-resistant (XDR) Salmonella Typhi has been emerged in Pakistan , The possibility for further blowout is of serious apprehension as lasting treatment options are cruelly limited. e report the outbreak of XDR Salmonella Typhi strains along with phenotypic and genotypic characterization of isolates.

Methods: Isolates were identified by phenotypic methods antimicrobial susceptibility testing was done by disc diffusion and final findings were confirmed by using Illumina whole genome nucleotide sequence data, All sequences were compared to the outbreak strain from Southern Pakistan and typed using the S. Typhi genotyping scheme

Results : All isolates were confirmed by a sequence analysis to harbor an IncY plasmid and the CTX-M-15 ceftriaxone resistance determinant.

Conclusion All isolates were of the same genotypic background as the outbreak strain from Sindh province. We report the first emergence of XDR S. Typhi in Punjab province of Pakistan confirmed by whole genome sequencing.

B026

Regulation of bacterial flagellar: a role for antibiotic resistance

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Abstract

Antibiotic resistance has been a serious challenge to the global health for many years. A comprehensive understanding of the molecular mechanisms driving this resistance, particularly the intrinsic factors, is crucial to develop a solution to that problem.

This study focuses on the transcriptional activator MarA, protein encoded by the *marRAB* locus of *Escherichia coli*. While MarA is primarily associated with activating the AcrAB-TolC efflux pump, responsible for active antibiotic export, our studies reveal its broader impact on bacterial physiology. In the current research we identified novel gene clusters influenced by MarA, particularly those involved in flagellar biosynthesis.

In *E. coli*, at least 14 operons, involved in flagellar regulation, and are expressed in a regulated "cascade". The operons are divided into 3 classes: the first two of are expressed in σ^{70}-dependent manner, the last is σ^{28}-dependent. MarA is traditionally recognised as a transcriptional activator of σ^{70}-dependent promoters, but our studies reveal the capability of MarA to activate σ^{28}-dependent promoters. Moreover, our investigation into the flagellar regulon has uncovered a new regulatory loop going beyond the traditional classification of flagellar genes.

These studies highlight the complex nature of flagellar regulation, offering new insights into the complexity of bacterial gene expression control and suggest that flagella, known for their role in bacterial motility, may play a role in adaptation under stressful conditions such as antibiotic exposure.

B027

The importance of genetic interactions in determining the pleiotropic effects of antibiotic susceptibility associated mutations in bacteria.

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Abstract

Single-step spontaneous mutation(s) which confer decreased susceptibility to an antibiotic are also often associated with pleiotropic effects which include bacterial fitness and collateral responses to other antibiotics. However, little is known about how the genetic background of different bacterial strains and species impacts these pleiotropic effects. Here, we investigated the impact of genetic background on the pleiotropic effects of mutations causing reduced antibiotic susceptibility between multiple strains of two species of Gram-negative bacteria. Single-step mutants with decreased susceptibility to ciprofloxacin were isolated from laboratory-derived and clinical strains of *Escherichia coli* and *Klebsiella pneumoniae*. The ciprofloxacin-selected single-step mutants isolated from three *K. pneumoniae* strains showed clinical levels of resistance to trimethoprim and nitrofurantoin. However, this clinical-level resistance was not present in the mutants isolated from one *K. pneumoniae* strain. Moreover, these collateral effects were also not present in any of the ciprofloxacin-selected *E. coli* mutants. Furthermore, the fitness of *E. coli* mutants was more variable as compared to the ciprofloxacin-selected mutants isolated from *K. pneumoniae*. These results suggest that the pleiotropic effects of mutation(s) associated with antibiotic susceptibility are influenced by genetic background at both the strain and species level. The outcome of this study highlights the importance of genetic interactions in determining the effects of mutations that reduce antibiotic susceptibility, which can impact the design of effective treatment strategies.

B028

The outcome of CRISPR-Cas9-directed AMR targeting depends on the genetic context of the target gene within E. coli ST131.

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Abstract

Antimicrobial Resistance (AMR) is a global health concern. CTX-M enzymes are a common group of Extended-Spectrum ß-lactamases (ESBL). The extraintestinal pathogenic *Escherichia coli* sequence type 131 (ST131), responsible for urinary tract and bloodstream infections, is often related to antibiotic treatment failure due to ESBL, specially blaCTX-M-15. Thanks to its sequence-specificity, CRISPR-Cas9 is a promising tool to target AMR. We explored the targeting of a chromosomally encoded blaCTX-M-15 in ST131 using a conjugatively delivered CRISPR-Cas9 cassette.

A CRISPR-Cas9 cassette targeting blaCTX-M-15 was conjugatively delivered to four ST131 human isolates. All isolates encoded blaCTX-M-15 chromosomally but in different genetic contexts: two isolates flanked by the insertion sequence (IS) Ecp1, and two isolates by several copies of IS26.

The delivery of the targeting CRISPR-Cas9 cassette led to a 2-4 orders of magnitude reduction in viable transconjugants, compared to a non-targeting control. Escapers carrying the targeting CRISPR-Cas9 plasmid were detected. ISEcp1-blaCTX-M-15 isolates presented a 10^{-5} escaping frequency, and the main escape mechanism were mutations in CRISPR-Cas9, likely causing CRISPR-Cas9 inactivation. In contrast, IS26-blaCTX-M-15 isolates presented a higher escaping frequency (10^{-3} to 10^{-4}), and the main escape mechanism was blaCTX-M-15 loss, leading to antibiotic resensitization of ST131.

Our study presents how CRISPR-Cas9-based targeting of AMR reduces the population size of ST131. Additionally, shows that genetic context of the target gene impacts the CRISPR-Cas9 outcome, through its effect on target gene loss rates. Overall, our study helps to understand the consequences of CRISPR-Cas9-based AMR targeting in realistic environments and its optimization as a tool against AMR.

B029

Biotechnological potential of *Bacillus dicomae* sp. nov., a bacterial endophyte isolated from *Dicoma anomala*

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Abstract

Plant species are known to host novel bacterial endophyte species, recognised as treasures of novel bioactive compounds with applications in various sectors such as pharmaceutical, chemical, agriculture, as well as food, leather, and food industries. Understanding these bacteria is thus of extensive relevance in microbiology and ecology and can reinforce multi-disciplinary applications of the discovered bioactive compounds. In this study, we aim to taxonomically characterise a novel bacterial endophyte species isolated from medicinal plant, *Dicoma anomala*; and explore its biotechnological potential through genomics. Our results indicate *Bacillus dicomae* sp. nov. genome encode a variety of genes and biosynthetic gene clusters which produce enzymes, and secondary metabolites valuable in the food, chemical, leather, and pharmaceutical sectors. This work highlights the benefit of exploring and isolating novel bacterial endophytes harboured in plants, moreover, the importance of using genomics to assess and understand the biotechnological applications of bacterial species is emphasised.

B030

Coupled Promoter-Terminators: a Missing Link in Transcription Regulation

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Abstract

Transcription in bacteria is controlled by precise start (promoters) and stop (terminators) signals. Promoters contain -10 and -35 regions that recruit RNA polymerase (RNAP) to the DNA and facilitate transcription initiation. Terminator sequences encode for hairpin structures in the nascent RNA that lever RNAP off the DNA to stop transcription. Promoters and terminators have long been thought of as separate entities. We report widespread coupling of promoters and terminators, in diverse bacterial species, on the basis of global transcription start and stop site mapping.

We have identified two potential functions for coupled promoter-terminators. First, in some cases, using the same DNA sequence for transcription initiation and termination saves space in crowded intergenic regions. Second, and more interestingly, genetic and biochemical tools show that coupling to promoters increases the efficiency of otherwise poor terminators. Mechanistically, enhanced termination is likely the result of collisions between terminating and initiating RNA polymerases at coupled promoter-terminator sequences.

Given the crowded nature of bacterial genomes, we suggest that coupled promoter-terminators may offer another method of genomic reduction, whilst being a fundamental level of transcription regulation which has previously avoided our attention.

B033

Understanding *Clostridioides difficile* diversity through genome sequencing

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Abstract

In recent decades hospital-acquired *Clostridioides difficile* infections has become a global concern. *C. difficile* is an important cause of nosocomial antibiotic-associated diarrhoea, and its ability to form spores poses a major challenge to infection prevention. In this study, we use whole-genome sequencing, using both long-read (Oxford Nanopore Technologies) and short-read (Illumina) platforms to describe the molecular epidemiology of *C. difficile* in a hospital in Birmingham, United Kingdom.

We collected strains of *C. difficile* from faecal samples, extracted DNA from them and performed DNA sequencing. We have so far performed long- and short-read sequencing on 13 isolates and will determine the phylogenetic relatedness of isolates and the presence of virulence and antibiotic resistance genes. We will also quantify the difference in performance of *C. difficile* genome sequencing, in terms of costs and sequence accuracy, of the Oxford Nanopore and Illumina platforms.

B034

What's on my food? Exploration of the *E. coli* sequence type diversity and population structure on retail foods from Norfolk, United Kingdom

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Abstract

Escherichia coli is used as an indicator organism for faecal contamination and antimicrobial resistance (AMR) on food. Understanding the diversity of *E. coli* lineages that contaminate retail foods, as well as the AMR genes present is important to understand potential risk to consumers. This study collected retail chicken, pork, salmon, prawns, and leafy greens in Norfolk, United Kingdom, isolating up to four *E. coli* isolates per sample. In total, 1067 *E. coli* isolates were collected and underwent whole-genome sequencing. Based on the phylogenomic tree, *E. coli* isolated from different food sources were phylogenetically heterogenous. When up to four *E. coli* isolates were taken from the same sample, all four isolates could be classed as different sequence types (STs). Significantly higher ST richness was observed from chicken compared to the other food commodities and a lower ST richness was observed from prawns compared to the other food commodities. Additionally, 13 different AMR drug classes were found spread across the 1067 genomes, seven of which were characterized as critically important by the World Health Organization (WHO), and five were classified as highly important. The five food commodities also had significantly different proportions of multidrug resistant samples (X^2 = 70.185, d.f. = 4, P-value = 2.08×10^{-14}), with *E. coli* from chicken having the highest proportion of isolates with multidrug resistance. This study highlights the importance of investigating more than one isolate per sample and provides insight into the potential reservoir of AMR genes on retail food.

B035

Using metagenomics to investigate associations of food production method with microbial ecology and antimicrobial resistance gene burden of retail foods.

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Abstract

The principles regarding antimicrobial use vary between different food production systems. These practices may affect the microbial populations as well as the prevalence and diversity of antimicrobial resistance genes (ARGs) found on food at retail. We aim to investigate how production methods influence the microbial and ARG ecology on retail food samples from organic, free-range, and conventional production systems.

A total of 146 beef, lamb, chicken, and leafy-green samples labelled as organic, free-range, or conventional were collected from retail outlets in Norfolk, England. Samples were host DNA depleted; the bacterial DNA was extracted and sequenced for taxonomic classification and ARG prediction.

Between 0 and 68 ARGs were identified per sample. Organic and free-range chicken were associated with significantly less ARG alpha diversity compared to conventional chicken. Conversely, organic lamb was significantly associated with higher ARG alpha diversity compared to conventional lamb. Food samples were taxonomically diverse, with a total of 418 genera identified. Food commodities harboured significantly different populations of bacteria and ARGs except for beef and lamb, which clustered together in both cases. While the between-sample beta diversity of ARGs were not significantly different between production methods on the same commodity, distinct populations of bacteria were identified between organic and conventional beef, with the latter characterised by an increased abundance of *Carnobacterium*.

These results demonstrate that different food production methods can be associated with the microbial composition and ARGs on retail food. Additional work is required to determine the factors accounting for these differences between food production methods.

B036

High *Campylobacter* diversity in retail chicken – epidemiologically important strains may be missed with current sampling methods

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Abstract

Campylobacter is recognised as a leading cause of bacterial gastroenteritis. Infections are underreported and outbreaks seldom identified, which may be partly due to limited isolate sampling from infections and potential food sources that host diverse *Campylobacter* populations, such as retail chicken.

In this study, 45 retail chicken samples were subjected to multiple culture method combinations, selecting up to 48 isolates per sample. The isolates were sequenced to determine the species and sequence type (ST) of each isolate and the distribution of STs utilised in simulation studies to determine the potential impact of high *Campylobacter* diversity on source attribution during outbreak investigations.

Thirty-nine (87%) samples were positive for *Campylobacter*, with 33% of samples containing two *Campylobacter* species (*Campylobacter jejuni* and *C. coli* or *C. jejuni* and *C. lari*) and 1-8 STs present on positive samples. Simulation studies showed that in order to identify 95% of the observed ST diversity, up to 87 isolates would need to be taken per sample. When each ST was in turn marked as a theoretical outbreak ST, simulations revealed that 26 isolates would be required on average for the probability of identification of the marked ST to reach 95%. Individual ST groups within samples also displayed up to 244 pairwise single nucleotide polymorphisms, indicating that the number of isolates required to capture *Campylobacter* diversity may further exceed current estimates. These findings indicate that selection of multiple isolates per sample in high diversity sample scenarios is necessary for accurate source attribution and outbreak investigation.

B037

Characterisation and optimisation of the rumen mcirobiome

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Abstract

One way to tackle the increased demand for animal protein, while mitigating environmental impacts of production, is to improve ruminant digestion efficiency by studying rumen microbes. Metagenomic and amplicon sequencing is one valuable approach to this, however the former can be subject to depth bias and fails to reconstruct repetitive sections of the genome, while the latter only amplifies a small fragment of the genome. Culturing can elucidate these microbes' functions and interactions, but is complex, and many microbes found in sequenced rumen fluid have not yet been cultured. To fully understand them it is best to use culturing and sequencing alongside each other. I have cultured bovine rumen fluid on a range of media at various dilutions. 16S rRNA sequencing identified 36 cultured genera from 8 phyla, with the different media supporting different communities that cluster distinctly by principal component analysis. On comparison to the operational taxonomic units found in the full rumen fluid, it was determined that 1.92% of the rumen microbiome had been cultured. Dilution-to-extinction and streak plating was then used to isolate these co-cultured microbes in pure culture. Around 14 pure cultures were obtained and classified to genus level based on 16S rRNA sequencing, including *Prevotella*, *Butyrivibrio*, and unclassified bacteria. Future work will include whole genome sequencing of the isolates and exploring their genomic and phenotypic characteristics, followed by manipulation of the rumen microbiome *in vitro* using these isolates while monitoring their effect on fermentation pathways and methane production, giving a better understanding of ruminant digestion.

B038

Genomic analysis of novel oral isolates of the human pathogen Fusobacterium nucleatum and an investigation of how virulence profiling and AMR marker screening could be exploited as clinical diagnostic tools.

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Abstract

Background

Fusobacterium nucleatum (F.nucleatum) is a Gram-negative organism naturally residing in the oral cavity and gastrointestinal tract (GIT) of humans. It has been associated with a number of human pathologies including a subset of colorectal cancers (CRC). Importantly, strains identified in the gastrointestinal tract and in CRC tumours have been found to be genetically identical to strains found in the mouth, hence investigation of the virulence of oral strains may provide insights into risk profileing for severe extra oral disease.

Methods

A selective media that enriches for *Fusobacteria* has been successfully used to isolate samples from healthy volunteers (ethical approval obtained). Nanopore whole genome sequencing was performed on DNA isolated from clinical isolates from the oral cavity of *F.nucleatum.* Sequences were assembled with Flye and annotation was performed using Bakta. Initial analysis has focussed on comparison of carriage, copy number and copy variation of 16s rRNA and virulence genes.

Results

F.nucleatum was isolated from both the saliva and gingival crevice of healthy volunteers. There is variation in the subspecies isolated from the different locations. Novel isolates show variation from genomes associated with archetype sub-species. *fadA* appears to be present with varying copy numbers across type strains and clinical isolates. The initial results validate the hypothesis that virulence factor carriage varies and therefore may influence the strains colonising the GIT.

Conclusion

Plaque and saliva screening of *F.nucleatum* has the potential to establish risk of developing a wide range of diseases, from periodontal disease to cancer.

B039

Fluctuations of health, disruptions to the microbiome in a *Campylobacter* and *Salmonella* gastroenteritis co-infection

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Abstract

Gastroenteritis is a global health challenge and dysbiosis of the microbiota impacts both the recovery of the patient. Individual pathogen infections have demonstrated disruptions to the microbiome, however, the microbial interactions in a co-infection case are not well understood. This case study investigated overall bacterial population structure and diversity of *Campylobacter* and *Salmonella* in a gastroenteritis clinical case during severe symptoms (S1), early recovery (S2) and full recovery (S3). Three stool samples were collected (S1-3) over 4 months. Samples were tested by PCR, culture and full metagenome DNA was extracted. Multiple isolates per sample and each metagenome was sequenced on Illumina Nextseq platforms.

Campylobacter jejuni ST-794 and *Salmonella* Enteritidis ST-183 were characterised in S1 and S2 only. Population diversity was observed in *Salmonella* antimicrobial resistance genotype and high clustering of *Campylobacter* genomes was identified.

Metagenome analysis identified the proportion of *Campylobacter* and *Salmonella* reads in the total metagenome was 0.54% and 0.21%, respectively at the severe symptom stage, while in early recovery, the proportions were 0.003% and -0.10% for *Campylobacter* and *Salmonella,* respectively. At full recovery, both pathogen proportions were below 0.001%. Low genome completeness limited pathogen characterisation in metagenomes. The bacterial population structure fluctuated between samples. Alpha diversity Shannon value was 0.47, 2.39 and 1.62 respectively while beta diversity expressed by PC1-2 plots depicted high dissimilarity between the three samples. This case study defined a changing pathogen population diversity and microbiome structure in response to a co-infection, underlying the potential impacts of pathogens on the gut microbiome.

B040

Optimising methods for Oxford Nanopore sequencing of bacterial epigenetic modifications.

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Abstract

Oxford Nanopore (ONT) sequencing technologies are among the few next- generation sequencing methods that are capable of acquiring both nucleotide sequence and epigenetic information simultaneously. The portability of the ONT MinION sequencing platform makes it particularly useful in non-canonical research settings. Given the increasing prevalence of antimicrobial resistance (AMR), and interests in researching the role for genome methylation in modulating AMR phenotypes, we aimed to develop a workflow to produce methylome data from Gram-negative bacteria using the MinION platform and R9.4.1 sequencing chemistry. Preliminary work made use of wild-type and methylase-deficient strains of *Escherichia coli,* with an overall ambition of establishing methods to produce figures and statistical reports representing the methylation state across the entire genome using genomic DNA extracted from *E. coli* using standard column-based commercial kits. Here, we describe the current state of this project, highlighting challenges surrounding epigenetic state base-calling, future opportunities for correlating AMR phenotypes with epigenetic profiles, and expanding upon the choice of laboratory strains used in this pilot study. We also highlight the opportunity to use epigenetic modification sequencing to study the effects of restoring methylase activity *in trans* into methylase-deficient strains using classical reverse genetics.

B041

Location, location, location – how does genomic context affect gene expression?

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Abstract

For all organisms, regulated gene expression that produces the appropriate complement of genes, at the right time, in the right place is vital. Changes in environment can occur rapidly, requiring efficient changes in gene expression. Control mechanisms have therefore evolved to allow living cells to replicate, produce phenotypes and adapt to varying environments. Genome position and organisation are important influences on gene expression in eukaryotes, but similar processes are poorly understood in bacteria. We do know that within bacteria, all genomic locations are not equally suitable for transcription of a given promoter, and the micro and macro structures of bacterial chromosomes are dynamic. In combination, these factors are major influences on transcription. Developing an improved understanding of the relationship between genome structure and gene expression will help us both understand genome evolution and provide information that can be applied in synthetic biology.

This project aims to provide an optimised method to study how genome structure influences gene expression in bacteria. RNA from massive libraries of *Escherichia coli* and *Salmonella* transposon mutants containing outward-facing inducible promoters was used to identify differences in expression from the same transcriptional unit across the genome. The impacts of changes in the environment (different growth phases and addition of antibiotics) on expression as well as chromosome supercoiling were determined. This approach provides a high-fidelity method to study the interdependency of gene expression, gene location and DNA topology.

B042

In silico investigation of the type VI secretion systems in clinical *Pseudomonas aeruginosa* isolates and effector prediction

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen that commonly infects people with Cystic Fibrosis (CF). The type VI secretion system (T6SS) is a molecular nanomachine that translocates effectors into target cells or the extracellular environment enabling intermicrobial interaction. *P. aeruginosa* encodes three T6SS clusters (H1-, H2- and H3-T6SS) and several orphan islands. Genetic diversity of T6SS-secreted effectors has been noted in reference *P. aeruginosa* strains but has yet to be explored in clinical isolates. Here, we perform a comprehensive bioinformatic analysis of the pangenome and T6SS effector genes in 52 high-quality clinical *P. aeruginosa* genomes isolated from CF patients. We confirm that the clinical CF isolate pangenome is open and principally made up of accessory and unique genes that may provide strain-specific advantages. Several characterised *vgrG* and *PAAR* islands were missing from numerous isolates and disruption of T6SS genomic loci through transposon, prophage, and mobile genetic element insertions. We identified the orphan *vgrG7* island in strain PAK and five clinical isolates that contains a gene encoding a putative Tle2 lipase effector. We also identified genes encoding eight new putative T6SS effectors with the following putative functions: cytidine deaminase, lipase, metallopeptidase, NADase, and pyocin. Our comprehensive *in silico* study of the *P. aeruginosa* T6SS exposes a level of genetic diversity at T6SS loci not seen to date within *P. aeruginosa,* particularly in isolates from people with CF and has now unlocked a path for *in vitro* characterisation of these mediators of intermicrobial competition which we are currently exploring in our lab.

B043

O-antigen polymerase polymorphisms in Salmonella: A mechanism for recovery following phage predation?

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Abstract

A key barrier to implementation of phage as antimicrobials is concern that bacteria readily become resistant to phage predation through spontaneous mutations affecting the expression of the bacterial cell-surface receptor used by phage to initiate infection. A common receptor used by many phages is the long chain O-antigen of LPS that forms a polysaccharide layer around Gram-negative bacteria. Since LPS is essential for virulence in many bacterial pathogens it has been proposed that resistant escape mutants are unlikely to be viable. We describe a genetic mechanism that may provide a means for the reversion of *Salmonella* that have become resistant to phage predation by a mutation that affects biosynthesis of LPS. Ancestral state reconstruction of a population of *Salmonella* Typhimurium that were either phage sensitive (DT8) or phage resistant (DT30) supported a model of bi-directional transformation of states with respect to phage sensitivity. GWAS indicated that phage resistant strains were significantly associated with a chromosomal deletion that resulted in the loss of the *wzy* gene. The Wzy O-antigen polymerase is required for elaboration of long chain O-antigen LPS used by phage to initiate infection. Deletion of wzy required two direct repeat sequences that flank the gene. A D*wzy* strain reverted to wzy+ genotype during co-culture with a donor wzy+ strain. We propose a model in which this mechanism provides a means for the reversion of bacteria that have become resistant to phage predation by a mutation that affects expression of the LPS receptor.

B044

Alignment-free clustering as an epidemiological tool for Mycobacterium tuberculosis recent transmission studies

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Abstract

Whole genome sequence (WGS) of *M. tuberculosis* employing a 5 SNP cut-off is a robust tool for surveillance investigations and detection of recent transmission events. This approach has shown many advantages in clinical and epidemiology studies. However, it was developed using isolates from low-incidence settings and requires significant computational resources, making it challenging to perform in many low-resource, high-incidence environments, where most tuberculosis cases occur. To address this problem, we explored alignment-free methods for clustering genomes to make the transmission tracking feasible in settings with limited computational resources.

In this study, we analysed over 300 clinical isolates from Rwanda using PopPunk (Population Partitioning Using Nucleotide *k*-mers), an alignment-free software for population analysis and clustering. Variable-length *k-mers* were applied to explore the core and accessory genomic variation between the isolates, and subsequently identify clusters of similar isolates. We then compared the core and accessory genome distance distribution with the standard SNP distance matrices to find any correlation.

Our analysis revealed that PopPunk has the potential to integrate *M. tuberculosis* data into real-time surveillance and prompt detection of emerging tuberculosis cases. We observed discrepancies in genetic distance between PopPunk and the gold standard WGS, suggesting further refinement and enhancement of the alignment-free tool for *M. tuberculosis* transmission studies is needed.

B045

An efficient pipeline for creating metagenomic-assembled genomes from ancient DNA in oral microbiome analysis

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Abstract

Metagenomic-assembled genomes (MAGs) are difficult to recover from ancient DNA (aDNA) as a result of substantial fragmentation, degradation, and contamination. These intricate features of aDNA raise concerns around whether bioinformatic tools intended for interpreting modern DNA are suitable for reconstructing ancient MAGs. This research investigates the extent to which MAGs can be built from such fragile and incomplete sequences and if they represent OTUs reliably to inform our understanding about disease in past populations. Our pipeline consists of a hybrid-scripted, multi-stage genomic binning, and user-friendly approach. We tested the validity of the pipeline by using 20 simulated datasets from publicly available genomes which we artificially modified using a combination of tools to better represent damage patterns observed in aDNA samples. We established that our workflow is adequate for retrieving oral bacterial and archaeal MAGs with promising authenticity from 105 archaeological dental calculus and tooth samples spanning 5000 years.

B047

Metabolic Profiling of *Salmonella* Paratyphi A Isolates Associated with the Bacterial Carrier State

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Abstract

Salmonella Paratyphi A is the dominant causative agent of paratyphoid fever, a human disease with an annual incidence of ~3.4 million cases. In addition to acute infections, individuals can harbour these bacteria asymptomatically as a carrier, a poorly characterised condition that has implications for transmission.

We examined the metabolic capacity of *S.* Paratyphi A isolates associated with chronic carriage, where infection persisted for >12 months, to assess their survival on alternative carbon sources *in vitro*. Initial isolates, collected by UKHSA, were compared to the most recent isolate from the same patient. Respiration was assayed across 95 carbon sources using the Biolog OmniLog system, and genetic differences between isolates were analysed using Illumina sequencing data from UKHSA.

Higher levels of respiration were observed in a patients' most recent isolate when utilising the majority of metabolised carbon sources, including a marked increase on pyruvate, and exclusive utilisation of D-ribose. SNP analyses highlighted longitudinal genetic variation within patients, revealing patients' latest isolates had developed potentially disruptive mutations in *fnr* and *adhE*, genes that encode a fumarate/nitrate reduction transcriptional regulator and bifunctional acetaldehyde-CoA/alcohol dehydrogenase respectively. Extended examination across a range of carrier isolates revealed one or both genes commonly mutated after extended incubation within a patient.

Enhanced utilisation of alternative carbon sources may aid *S.* Paratyphi A's survival in the host and could be facilitated by alterations to the global regulator *fnr.* By understanding changes in the metabolic capacities of isolates during persistent infections, we can better understand the carrier state and its role in transmission.

B048

Soil Microbiome Variation due to Flooding: A Comparison of 16s rRNA amplicon Long-read and Shotgun Metagenomic Sequencing.

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Abstract

According to the World Health Organisation, climate change represents one of the most significant threats to global health. As a result of climate change, increased flooding events have been recorded across the globe. Anaerobic conditions caused by waterlogging have been shown to reduce the populations of beneficial aerobic bacteria and cause changes in the soil microbiome.

Eight site matched flooded and non-flooded soils were sampled across Ireland. DNA was extracted from all samples and extensive quality control procedures were performed. Long-read 16s rRNA amplicon sequencing, using Oxford Nanopore Technology, and Shotgun metagenomic sequencing were performed on the same samples. In-depth bioinformatic analysis was performed on the sequencing data, allowing for the identification of the soil microbiome composition and comparison of the two methods.

Changes in the balance of soil bacterial communities, many of which are vital for nutrient cycling and soil fertility, were identified. Phyla such as Acidobacteria were significantly increased in flooded soil, whereas Proteobacteria and Bacteroidetes were decreased. In addition, similarities and differences between the two sequencing methods, such as data outputs and cost/time effectiveness were revealed. Both sequencing methods showed significant bacterial microbiome variation between flooded and non-flooded soils.

B049

Temperate phages of cystic fibrosis and non-cystic fibrosis bronchiectasis drive physiological changes in Pseudomonas aeruginosa, during exposure to the antibiotic meropenem.

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Abstract

Pseudomonas aeruginosa (Pa) is an opportunistic respiratory pathogen in chronic respiratory disease including Bronchiectasis (BR) and Cystic Fibrosis (CF). Temperate bacteriophages, integrated into the bacterial chromosome of Pa, can carry genes that aid selection in the lung, driving evolution. We here characterise the genomes of 4 phages that mobilise from CF/BR related Pa and subvert function through their Pa host range.

Four lysogens of PAO1 were created using phages induced from CF and BR Pa isolates. Lysogeny was confirmed by sequencing and aligning to the naïve PAO1 host.

We determine that temperate phages offer a selective advantage to PAO1. Cellular physiology is altered with lysogeny that allows the bacterium to develop an increase in antibiotic resistance to meropenem moving from tolerance to resistance when comparing to the naïve wt bacterium. Lysogens moving to resistance were assessed for SNP's, metabolic shift and differential RNA expression during exposure to meropenem at the set concentrations. This study illustrates that Pa temperate phages, isolated from the lung, drive adaptation and subversion linked to metabolism that can lead to bacterial fitness, potential virulence, and reduced AMR susceptibility. Importantly development towards AMR does not seem to be linked to classified AMR genes. Moreover, is likely linked to subversion of cell physiology under early selective pressure.

B050

Genomic epidemiology of antimicrobial resistance and virulence plasmids in *Escherichia coli* and *Klebsiella pneumoniae* from One Health settings

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Abstract

Plasmids are major drivers of bacterial evolution, and recent advances in long-read sequencing make it possible to generate complete assemblies of plasmid genomes for large population samples. Here we apply this technology to the critical priority pathogens *Escherichia coli* and *Klebsiella pneumoniae* from humans, animals, and the environment, to determine how frequently hybrid plasmids emerge that possess both antimicrobial resistance and virulence traits.

We utilised *E. coli* and *K. pneumoniae* genome sequence data from a large One Health study conducted across Thailand, generated by the OH-DART consortium. We focused on strains carrying the plasmid-mediated virulence locus *iuc5*, which is associated with *E.coli*, and used long-read sequencing to generate hybrid assemblies of 68 *E. coli* isolates and 2 *K. pneumoniae* isolates harbouring the *iuc5* locus. Resfinder and Prokka were used to identify plasmids with acquired resistance genes and the *iuc5* locus, respectively.

In total we identified 230 circular plasmids from *E. coli* strains harbouring the *iuc5* locus. Of these plasmids, 70 carried iuc5, and 68 of these plasmids contained 1 or more antimicrobial resistance genes. These plasmids were isolated from human community samples, hospital samples, fresh markets and across several different chicken, duck, and fish farms. We also identified two *iuc5*-harbouring plasmids in *K. pneumoniae* isolated from hospital samples.

Here we report that plasmids harbouring the *iuc5* locus and antimicrobial resistance genes are widespread across different ecological sources, emphasising the importance of monitoring virulence alongside antimicrobial resistance surveillance within a One Health context.

B051

The evolutionary dynamics of the β-lactamase locus and its relationship with methicillin resistance in Staphylococcus aureus

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Abstract

The β-lactamase (bla) locus confers resistance to penicillin in Staphylococcus aureus. It consists of a structural gene (blaZ) that is regulated by a repressor (blaI) and signal transducer (blaR). Previous studies have found evidence that blaI and blaR additionally regulate the expression of mecA, a gene that confers resistance to a broad-spectrum of β-lactam antibiotics, including methicillin. Despite this, little is known about the drivers of the gain and loss of mobile genetic elements (MGEs) that carry the bla locus, and how this influences the gain and loss of mecA. Here we present the first comprehensive characterisation of the MGEs carrying blaZ in a S. aureus clonal complex (CC). Using a collection of 1181 genomes, we describe the diversity and dynamics of MGEs carrying blaZ in CC398, the dominant livestock-associated MRSA in Europe. We find that while blaZ is rarely absent from either the MRSA or methicillin-susceptible, MSSA, populations of CC398, this superficial stability is underpinned by the carriage of several different MGEs. Using ancestral state reconstruction, we infer the dates of acquisition of each MGE and contextualise them based on other events in the lineage. We identify a correlation between losses of mecA and blaZ, indicating an important selective benefit of blaZ retention after mecA acquisition. We further find that an association between the presence of blaZ and mecA is observed broadly across S. aureus, and in other staphylococci. These results suggest that the bla locus plays an important role in the evolution and persistence of broad-spectrum β-lactam resistance in staphylococci, and that its loss may promote the reversion of MRSA to MSSA.

B052

Allelic variation as a driver of multidrug resistant *Escherichia coli*

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Abstract

Multi-drug resistant (MDR) *Escherichia coli* is a leading cause of invasive disease in humans and animals. *E. coli* can be divided into sequence types (STs) based on MLST. Clones from a small number of STs are responsible for the majority of invasive human MDR *E. coli* infections globally. These pandemic MDR clones have emerged on multiple occasions over varying timeframes, highlighting the importance of studying the evolutionary pathways that led to their success.

This research examines unique patterns of selection in MDR *E. coli* by comparing the core genomes of diverse *E. coli* lineages. The aim of this work is to identify and characterise genes with hyper-conserved and hyper-variable sequences, identifying genes that come under positive and purifying selection in pandemic clones compared to other lineages.

A curated collection of 20,473 *E. coli* genomes was interrogated by creating ST-specific core genomes. The dataset spans the phylogenetic diversity of the species and includes representatives of 21 STs. Single nucleotide polymorphisms (SNP) were called against each gene in each core genome. The frequency of SNP sites in each gene was calculated and compared between STs, with a focus on differences within and between pathogenic lineages (MDR and non-MDR) and non-pathogenic lineages.

We identified hyper-conserved genes, indicative of purifying selection, and hypervariable genes, suggesting selective pressures including immune evasion. This therefore allows us to identify genes that come under unique selective pressures in MDR clones of *E. coli* compared to drug susceptible lineages, furthering our understanding of the evolutionary trajectory of MDR pathogens.

B053

Genomics of Malawian *Staphylococcus aureus* keratitis isolates

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Abstract

Purpose

S. aureus is a major microbial keratitis (MK) pathogen worldwide. We used whole genome sequencing to identify key virulence factors in Malawian *S. aureus* keratitis isolates.

Methods

Seven *S. aureus* strains from MK patients were sequenced and characterised using multi-locus sequence typing (MLST). The assembled *S. aureus* isolates were compared to a custom virulence factor database consisting of 31 virulence genes associated with ocular *S. aureus* infection.

Results

The most represented sequence types in the seven Malawi *S. aureus* keratitis isolates were ST9572 (n=2) and ST10011 (n=2). Of the adherence genes studied, clumping factor A (*clfA*), fibronectin-binding Protein A (*fnbA*) and serine-rich surface protein *(sraP*) were present in all isolates. Numbers of evasion genes varied greatly between the strains. The cysteine protease genes (*scpA*, *sspB* and *sspA*) were found in all isolates. Staphylococcal enterotoxins were found in all but one of the isolates with varying profiles. Of the invasion genes studied, gamma and delta-hemolysin genes were found in all seven isolates. The *lukF*-PV and *lukS*-PV gene encoding Panton-Valentine leucocidin (PVL) were present in 3/7 (43%) and 2/7 (29%) of isolates respectively. The methicillin resistance gene (*mecA*) was not identified in any isolates.

Conclusions

This is the first study to investigate *S. aureus* virulence factors in MK in Sub-Saharan Africa. There was a high diversity of *S. aureus* sequence types and virulence genes that have been associated with ocular *S. aureus* isolates. Importantly, there was a high prevalence of PVL, a virulence factor known to be associated with poorer outcomes.

B054

Isolation and characterisation of Shigatoxigenic *Escherichia coli* (STEC) serotype O157:H7 from Scottish sheep

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) serotype O157:H7 is a priority zoonotic pathogen which, in humans causes gastrointestinal infections, haemorrhagic colitis and haemolytic uremic syndrome. Scotland has more human cases per head of population than any other part of the United Kingdom. Previous studies have demonstrated how strains from cattle, wild deer and fresh beef mince on retail sale in Scotland relate to Scottish human clinical cases. Here, we examined another important Scottish livestock ruminant species, sheep, to identify the diversity of STEC strains they carry. To do this, 875 faecal samples were collected from Scottish sheep during 2022/23; some were collected from the environment during the periparturient period and others collected post-mortem, at abattoirs. STEC was isolated from the samples using enrichment procedures and immunomagnetic separation followed by recovery on CT-SMAC and Chromocult coliform media. Fourteen of the samples each yielded a single presumptive STEC O157 isolate with thirteen from abattoir faecal samples and a single isolate from the field flocks. The isolates were confirmed using an agglutination test that targets the STEC O157 lipopolysaccharide and by whole genome sequencing. Genome analyses showed that all the isolates are STEC O157:H7, ST11 and encode the *eae* and *stx* genes (except one isolate which lacked the *stx* genes). Genotyping and phylogenetic analyses revealed that all the isolates cluster closely with human isolates. Our data supports the carriage of STEC O157:H7 among Scottish sheep and the genomic relationship of the isolates with those from humans suggest a potential for inter-host transmission.

B055

In vitro evolution of *Enterococcus faecium* in the presence of vancomycin

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Abstract

The Gram-positive bacterium *Enterococcus faecium* is an important cause of nosocomial infections. There are limited treatment options for *E. faecium* infections, particularly when acquired transposons confer vancomycin resistance. To identify whether *E. faecium* can evolve additional mechanisms to tolerate vancomycin, the vancomycin-susceptible *E. faecium* strains E1162 and E2560 were evolved *in vitro* in a range of vancomycin concentrations above and below the minimum inhibitory concentration (MIC) in three independent biological replicates. Growth was assessed daily and the culture that grew at the highest concentration of vancomycin was inoculated into fresh media with increasing vancomycin concentrations. This was repeated for 11 d. We consistently observed evolved populations growing at increased concentrations of vancomycin (from 0.5 ug/ml to 1 ug/ml vancomycin). Genome sequencing of evolved strains revealed single nucleotide polymorphisms (SNPs) in genes encoding the WalKR/YycFG two-component system (TCS), which is involved in regulation of peptidoglycan biosynthesis. Both E1162 and E2560 had evolved replicates exhibit non-synonymous SNPs in *walK*, which encodes the histidine kinase in the WalKR TCS. A further E1162 evolved replicate had a SNP in *yycI*, which encodes a protein predicted to regulate WalK activity. Morphological changes were observed in evolved strains in comparison to parent strains, particularly an increase in chain length and a statistically significant increase in cell wall thickness. As mutations in *walKR* have been associated with increased tolerance to antibiotics such as daptomycin and vancomycin in Staphylococcus aureus, we postulate that exposure to vancomycin at sub-MIC may cause increased tolerance to last-resort antibiotics in *E. faecium*.

B056

Phenomics of antimicrobial resistance in Streptococcus uberis

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Abstract

Streptococcus uberis is a common and poorly controlled cause of intramammary infection in dairy cattle, responsible for a high proportion of bovine mastitis worldwide. Control of bovine mastitis still relies on the use of therapeutic and prophylactic antibiotics. However, antimicrobial resistance (AMR) in mastitis pathogens including *S. uberis* typically remains low, although exceptional incidence of resistant isolates have been reported.

In this study, 250 *S. uberis* isolates from the UK (1997 – 2022) were sequenced and phenotyped against 11 clinically relevant therapeutic agents. These data were used to conduct genome wide association studies (GWAS), analysed using *pyseer*, to identify sequences associated with resistance to erythromycin and pirlimycin.

All k-mers identified in the GWAS of erythromycin resistance mapped to putative Mu-like bacteriophage regions that harboured multiple antimicrobial resistance genes including *ermB,* which had the highest p-value. Similarly, 94.4% of pirlimycin associated k-mers mapped to putative Mu-like bacteriophage regions and k-mers with the highest significance (p-value) mapped to *aadK*, a gene associated with reduced susceptibility to streptomycin. However, not all isolates with high minimum inhibitory concentrations could be explained by mobile genetic elements suggesting other mechanisms of resistance exist in *S. uberis*. This study highlights the importance of mobile genetic elements in *S. uberis* multi-drug resistance and provides further evidence of the role of mobile genetic elements in the transmission of sequences involved in AMR.

B057

Genomic Analysis of uropathogenic E. coli strains in Egypt.

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Abstract

E. coli is the most common cause of urinary tract infections (UTIs). The emergence of multidrug resistant (MDR) is an emerging and serious public health problem resulting in treatment failure, increased length of hospital stay and high mortality. Our study aimed to characterise the resistome of 10 *E. coli* strains associated with UTIs in Egypt. Whole genome sequencing (WGS) was carried out using an Illumina MiSeq platform and *in-silico* analysis revealed resistance against beta-lactams due to the presence of specific genes of the β-lactamase extended spectrum (ESBL) including *bla*SHV-12*,* *bla*CTX-M-15*,* *bla*TEM-1B*,* *bla*NDM-5*,* *bla*NDM-19, *bla*DHA-1, and *bla*CMY-2. In addition to resistance to other antibiotic classes including quinolones, aminoglycosides, folate pathway antagonists, fosfomycin, macrolides, tetracyclines, quaternary ammonium compounds and amphenicol. None of *E. coli* isolates showed resistance to the last-resort antibiotic; colistin. Genomic analysis of the clinical *E. coli* strains also revealed bacterial isolates harbour several virulence factors that help *E. coli* overcome host defences and colonize or invade the urinary tract including the aerobactin system, hemolysin, K capsule, resistance to serum killing and adhesins such as P fimbriae and type 1 fimbriae. A comprehensive analysis of MDR *E. coli* strains provided by WGS detected variable antimicrobial resistance determinants and virulence factors of recognized importance in the pathogenesis of UTI. A longer-term aim of our project is to elucidate the diversity of UPEC strains associated with UTIs from different countries in the world that will help enhancing our understanding of *E. coli* infections in UTIs.

B059

Ordering taxa in image convolution networks improves microbiome-based machine learning accuracy

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Abstract

The human gut microbiome is associated with a large number of disease etiologies. As such, it is a natural candidate for machine-learning-based biomarker development for multiple diseases and conditions. The microbiome is often analyzed using 16S rRNA gene sequencing or shotgun metagenomics. However, several properties of microbial sequence-based studies hinder machine learning (ML), including non-uniform representation, a small number of samples compared with the dimension of each sample, and sparsity of the data, with the majority of taxa present in a small subset of samples. We show here using a graph representation that the cladogram structure is as informative as the taxa frequency. We then suggest a novel method to combine information from different taxa and improve data representation for ML using microbial taxonomy.

iMic (image microbiome) translates the microbiome to images through an iterative ordering scheme, and applies convolutional neural networks to the resulting image.

We show that iMic has a higher precision in static microbiome gene sequence-based ML than state-of-the-art methods. iMic also facilitates the interpretation of the classifiers through an explainable artificial intelligence (AI) algorithm to iMic to detect taxa relevant to each condition. iMic is then extended to dynamic microbiome samples by translating them to movies.

B061

A comparative genomics approach to analyse antimicrobial resistance trends in ESBL-Enterobactericeae among Tanzanian neonates recruited into a probiotic clinical trial, ProRIDE

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Abstract

Probiotics may be a promising tool to improve health outcomes of infants, however their effect on the mobility, persistence, and co-mobilisation of antimicrobial resistance genes (ARGs) is relatively unexplored. The ProRIDE randomised clinical trial recruited 2000 Tanzanian neonates to determine the effect of a probiotic blend on health outcomes and Extended Spectrum Beta-Lactamase producing *Enterobacteriaceae* (ESBL-*E*) carriage in the gut. This sub-study aimed to analyse antimicrobial resistance trends in ESBL-*E* using comparative genomics.

One rectal swab per patient was taken two weeks after treatment completion. Within 500 swabs, 97 and 31 ESBL *E. coli* and *K. pneumoniae*, respectively, were isolated then sequenced by MicrobesNG using Illumina sequencing (2x250bp paired-end). Genomes were queried against SRST2-ARGANNOT, ISFinder, PlasmidFinder, PubMLST using ARIBA. *de-novo* assemblies were constructed by Shovill and annotated with Prokka. Phylogenies were calculated using IQTREE and visualised in iTOL, supplemented with metadata. ARG co-occurrence and genomic structures were analysed and visualised using R.

Both species displayed diversity in ARGs, plasmids and sequence types, however 85.5% of genomes had $bla_{CTXM\text{-}15}$, both putatively plasmid-borne and chromosomal. Between clinical groups, co-occurrence analysis revealed differences in potential $bla_{CTX\text{-}M\text{-}15}$ co-mobilisations within *E. coli* and greater entropy of co-occurrences within the intervention group. Minimal differences were found among *K. pneumoniae,* partially attributable to a higher proportion of ESBL bla_{SHV} variants and subsequent sample size. No significant difference in ARG frequency were observed in either species.

Research is ongoing to validate these predicted ARG co-mobilisations and their mechanisms of intracellular transposition. Understanding the interaction of live organisms with the resistome would better inform their suitability for neonatal health.

B062

Coagulase-negative strains of Staphylococcus aureus identified in New Zealand dairy cows

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Abstract

The dairy industry is a pasture-based seasonal industry in New Zealand, and minimising animal disease is essential for maintaining productivity. Mastitis incurs significant economic costs, amounting to approximately $NZ280 million annually. One cause of mastitis is the contagious bacterium *Staphylococcus aureus* (*S. aureus*), accurate and reliable identification of this bacterium is crucial to treat infected cows effectively.

To better understand the population distribution of bovine *S. aureus* and non-aureus staphylococci (NAS) in New Zealand, our study collected and performed whole genome sequencing (WGS) on 2010 *Staphylococcus* isolates. Isolates were obtained from aseptic quarter samples and bulk tank milk from 387 farms. WGS identified 913 isolates as *S. aureus*, phenotypically *S. aureus* is typically β-haemolytic, catalase and coagulase positive. Subsequent isolate phenotyping led to the discovery of 87 atypical *S. aureus* isolates with a negative rabbit plasma coagulase result and pink/mauve colonies indicative of *S. aureus* on selective chromogenic media (CHROMagarT *Staphylococcus*). Atypical isolates were confirmed as *S. aureus* via MALDI-TOF.

Genomic analysis revealed that 90% of the atypical *S. aureus* belonged to the bovine-adapted clonal complex 151 (CC151). Investigation of the staphylocoagulase gene revealed two novel coagulase gene variants, likely resulting in a non-functional coagulase product possibly accounting for the negative rabbit plasma result. No reports of coagulase-negative variants of bovine *S. aureus* exist in New Zealand. Further investigation is required into the epidemiological characteristics of these isolates. Understanding the role of atypical *S. aureus* strains is vital in evaluating the true prevalence of *S. aureus* in the bovine population.

B063

Reverse antimicrobial engineering: targeting optimal ribosomal protein subunits

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Abstract

Antimicrobial-resistant (AMR) pathogens are a major threat to human and animal health. The decline in the efficacy of antimicrobials has led to major investment in identifying compounds that can disrupt or destroy bacterial cells. Success depends upon identifying optimal foci for directed pathogen killing. These target foci would need to be ubiquitous in bacteria, different from eukaryote hosts to avoid cross-reactivity, and fundamental to cell replication. Therefore, there is no better target than the engine of protein synthesis, the bacterial ribosome. Ribosomal protein subunits (*rps*) genes are an important target for existing antimicrobials, including aminoglycosides, tetracyclines, macrolides, and chloramphenicol. However, the selection of novel ribosomal protein targets is rarely systematic and does not take into account the variation in the *rps* genes between bacterial strains and species. Here we analyse sequence variation among the 53 *rps* genes in genomes from multiple bacterial species. *In silico* predictions of protein interactions with multiple drug classes used a novel bioinformatics, phylogenetics and structural biology pipeline. This incorporated analysis of structure-activity relationships between drugs and ribosomal protein subunits and the role of *de novo* mutation and horizontal gene transfer in the spread of AMR between strains and species. By beginning analyses with natural bacterial populations, we reverse typical approaches aimed at understanding the mechanisms of resistance in laboratory strains. This provides a new approach for the systematic discovery of novel therapeutic targets to combat AMR.

B064

The insertion sequence-mediated disruption in the capsule locus of *Acinetobacter baumannii* causing increased virulence in *Galleria mellonella*

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Abstract

Background: *Acinetobacter baumannii* is a Gram-negative opportunistic human pathogen expressing multiple virulence factors such as biofilm formation, secretion systems or capsular polysaccharide, encoded by a locus (KL), which varies among different isolates. We have examined the role of a particular KL gene in virulence.

Methods: We identified an insertion sequence deleting a part of the acetyltransferase-encoding gene within the KL. The complete acetyltransferase-encoding gene from an isolate encoding the same allelic variant was cloned into a plasmid vector and electroporated into the host cell. We have assessed the virulence of the wild-type, the plasmid-complemented and the strain with an empty plasmid vector using *G. mellonella* model. The growth rate in the rich medium was assessed to determine the fitness cost of the plasmids on the host cells.

Results: The bioinformatic analysis revealed the presence of IS*Aba26* and IS*Aba31* in the KL of the clinical isolate of *A. baumannii*, with IS*Aba31* deleting part of the acetyltransferase-encoding gene. While the wild-type and the strain with the empty plasmid vector were highly virulent, killing 100% of *G. mellonella* larvae within 48h, the strain with plasmid-complemented acetyltransferase-encoding gene was significantly impaired in virulence, rendering it avirulent. The growth curves showed no significant impact of the plasmid encoding acetyltransferase on the host cell.

Conclusion: We have identified a native gene, which negatively influences the virulence of *A. baumannii* encoding specific KL type. This observation could shed light on virulence mechanisms *in vivo* and could be explained by the selection of the virulent variant of the strain within the patient.

B065

Temperature-dependent regulation of adherence and virulence in Streptococcus pneumoniae

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Abstract

Streptococcus pneumoniae (SPN) is a Gram-positive commensal bacterium of the human upper respiratory mucosal surfaces. It exhibits opportunistic pathogenicity, causing infections ranging from non-invasive to severe, including community-acquired pneumonia, meningitis, and sepsis. SPN possesses diverse virulence factors and is naturally competent, capable of genome remodeling that can lead to vaccine escape and the emergence of antibiotic resistance. Thriving across temperatures from 33 °C in the nasopharyngeal cavity to 37 °C in deeper host niches, or 39 °C during fever, it transitions from commensal to pathogenic, in part, through the action of bacterial signal-transduction systems that respond to environmental cues.

In this study, we identified temperature-dependent post-transcriptional regulation of genes encoding an SPN two-component signalling system. Control of protein production is achieved through the action of an RNA thermoregulatory element within the 5´-untranslated region (5´-UTR) of the *ciaRH* operon. We investigated the effects of temperature-dependent regulation of this system. Using a Δ*ciaRH* strain, and mutants with engineered 5' UTR sequences that yielded translation-restrictive ($ciaRH^{(p:close)}$) or translation-permissive ($ciaRH^{(p:open)}$) mRNA secondary structures, we examined the effects of post-transcriptional regulation of *ciaRH* on SPN phenotypes. Here, we will present the results of studies of growth, biofilm formation, cell wall content, beta-lactam resistance and virulence in wild type SPN and those in which the CiaRH two-component system has been uncoupled from thermal regulation.

B066

Determination of *Escherichia coli* adhesiomes with adhesiomeR

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Abstract

Adhesins are a key factor enabling host colonization and pathogenesis of bacteria, including *Escherichia coli*. Therefore, they are promising targets for intervention strategies such as vaccination and anti-adhesion treatments. Antimicrobial-resistant (AMR) *E. coli* strains are now a leading cause of death, necessitating novel treatments of *E. coli* infections. However, to date no resources have been dedicated to the detailed characterization of *E. coli* adhesins in (meta)genomics data.

To enable characterization of *E. coli* adhesiome, i.e. the complete adhesin repertoire, we developed adhesiomeR, a powerful framework that enables qualitative inspection of the adhesin repertoires. It is based on the most comprehensive manually curated set of 525 *E. coli* adhesin genes grouped into 102 systems. We used 15,590 *in silico* pathotyped *E. coli* genomes to define adhesin profiles present in the *E. coli* population based on genes found by adhesiomeR. We identified 7,038, 4,770 and 1,443 unique profiles when considering all, fimbrial and nonfimbrial adhesin genes, respectively. Further clustering of adhesin profiles revealed associations of certain adhesin genes with pathotypes. We evaluated adhesiomeR using 354 ETEC strains with known profiles of 19 adhesin systems and demonstrated that it achieves 98% accuracy compared to experimentally validated analyses.

AdhesiomeR fills the gap in the field of virulence-associated factors by focusing specifically on adhesins and offers valuable cost-effective insights into *E. coli* adhesiomes. Results obtained with adhesiomeR can assist experimentalists in characterizing *E. coli* strains relevant to human and animal health and guide the development of novel treatment strategies against AMR and pathogenic *E. coli*.

B067

Changes in the rate of genomic variation during the transition of acute to chronic infection

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Abstract

Background: *Salmonella enterica* serovar Agona (*S*. Agona) has been increasingly recognised as a prominent cause of gastroenteritis. This serovar is a strong biofilm former that can undergo genome rearrangement and enter a viable but non-culturable state whilst remaining metabolically active. Similar strategies are employed by *S*. Typhi, the cause of typhoid fever, during human infection, which are believed to assist with the transition from acute infection to chronic carriage. Here we report *S*. Agona's ability to persist in people and examine factors that might be contributing to chronic carriage.

Methods: A review of 2,233 *S*. Agona isolates from UK infections (2004-2020) and associated carriage was undertaken, in which 1,155 had short-read sequencing data available. A subset of 207 was selected from different stages of acute and persistent infections within individual patients. The subset underwent long-read sequencing and genome structure (GS) analysis, as well as phenotyping assays including carbon source utilisation and biofilm formation. Associations between genotypes and phenotypes were investigated to compare acute infections to those which progress to chronic.

Results: GS analysis revealed the conserved arrangement GS1.0 in 195 isolates, and 8 additional GSs in 12 isolates. These rearranged isolates were typically associated with early, convalescent carriage (3 weeks – 3 months). Our SNP rate analysis also showed an increase in SNP variation during this time of infection.

Conclusions: We believe this increase in genome-scale and SNP variation reflects a population expansion after acute *S*. Agona infection, potentially reflecting an immune evasion mechanism which enables persistent infection to become established.

B068

Everything, Everywhere All at Once : an annotation-independent pipeline for Transposon Insertion Sequencing

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Abstract

Transposon Insertion Sequencing (TIS) identifies genes that are essential for growth of bacteria under varying conditions. Pools of insertion mutants are grown, then sequenced to locate and quantify the number of insertions in surviving cells; the insertion frequency indicates mutant fitness. One major limitation of TIS is the lack of precise annotation for many bacterial genomes. This hinders current analysis pipelines which look at expected vs. actual insertion within genes. To overcome this, we have developed a new annotation-independent pipeline based on changepoint detection algorithms.

Annotation-independent analysis is less restrictive and more informative than focusing only on annotated genes as non-coding genomic regions can contribute to survival and growth under stresses. Changepoint analysis enables us to divide the genome into regions depending upon insertion rate. An insertion rate lower than expected suggests essentiality and higher than expected is important for regulatory pathways; rarely investigated outside of expression studies.

Important considerations include normalisation or adjustment for sequence, DNA access and procedural bias. Segmentation based on insertion rates remained consistent even if the reference genome annotation changed, meaning annotation overlaid for a reference genome will allow historical experiments to be updated.

Furthermore, downstream analysis can assign functions to the identified regions to highlight essential functions and processes required for survival, particularly in the case of bifunctional genes. Using this approach could improve genome annotation for poorly annotated genomes, with subsequent positive impacts upon genome-wide analysis methodologies including metabolic modelling, functional genomic studies, and understanding of regulation within genomes.

B069

Large-scale genomic analysis of Klebsiella pneumoniae epidemic ST11 and ST15 lineage

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Abstract

Klebsiella pneumoniae (Kp) contributes to hospital and community-acquired infections, primarily because of its capacity for acquiring resistance and virulence factors. Recently, Kp ST11 and ST15 have emerged as epidemic lineages in China. However, despite the increasing carbapenem resistance and virulence in these lineages, the reason behind the evolution dynamics and the route of transmission remains poorly understood.

Using over 2000 publicly available isolates (1157 of ST11 and 848 of ST15), our study focused on the core genome (CG) tree, geographical distribution, hierarchical clustering, and key phenotypic attributes such as antibiotic resistance and virulence.

We constructed the core genome (CG) tree and clustered ST11 and ST15 data using hierarchical clustering. Our analysis suggests specific hierarchical groups which are rapidly emerging in Asia, particularly in China. These clonally expanded clusters in China, exhibit additional resistance to tetracyclines, chloramphenicol, trimethoprim,
and rifamycins. The rmp locus, which linked to hypermucoviscosity, was also identified in the ST11 clonal expansion cluster together with the additional antibiotic resistance. Whilst ST11 and ST15 is predominantly found in Asia, we found that there are specific hierachical groups which are globally disseminated.

Our study highlights the geographically constrained evolution of ST11. While dominant clones within ST11 and ST15 are rapidly emerging in China, their global dissemination indicates a potential worldwide spread of antibiotic resistant and hypervirulent lineages.

B070

Characterisation of rifampicin resistant *Stenotrophomonas maltophilia* Sm314 mutants isolated from an in vitro human lung epithelium infection model

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Abstract

Stenotrophomonas maltophilia, a Gram-negative environmental bacterium, is increasingly recognised as a nosocomial infection agent due to expression of various virulence factors, inherent antimicrobial resistance and biofilm-forming capability. It commonly causes pneumonia, particularly in immunocompromised or cystic fibrosis (CF) patients. Co-isolation with the fungal opportunist *Candida albicans* suggests a potential enhancement of antimicrobial-resistant bacterial co-colonisers.

Our study investigated the impact of environmental conditions of the lung epithelium and *C. albicans* co-infection on the *S. maltophilia* CF isolate Sm314. We hypothesized that CF lung conditions may induce or select mutations in *S. maltophilia* Sm314, promoting development of antimicrobial resistance. Using a Calu-3 lung epithelial layer model in transwell inserts at the air-liquid interface (ALI), we conducted single and mixed infections over seven days. Eight clones, exhibiting increased rifampicin resistance under various conditions, were selected for further analysis through whole genome sequencing, phenotypic assays, and molecular computational simulations.

Co-infection with *C. albicans* does not appear to promote the development of rifampicin-resistant *S. maltophilia* Sm314 mutants, but selected clones showed increased rifampicin minimum inhibitory concentration and heritability of resistance without selective pressure. Whole genome sequencing revealed mutations in *rpoB*, encoding β-subunit of RNA polymerase, in all clones. Modelling the RpoB protein of a mutant with an unusually large deletion in rifampicin-resistance-determining region I, revealed a dramatic conformational change in the rifampicin binding site, while maintaining the ability to transcribe RNA.

In summary, we found and characterised rifampicin-resistant mutants from *S. maltophilia* biofilms in ALI lung epithelial cell cultures under as yet unidentified selection conditions.

B071

Genomic studies of honeybee symbionts reveal regulatory systems for antibiotic production and biofilm formation

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Abstract

Apilactobacillus kunkeei has been isolated from the honey crop and food sources of honeybees. *A. kunkeei*'s genomes are 1.5-1.6 Mb and some isolates contain plasmids for the synthesis of kunkecin A, an antibiotic that inhibits the growth of the honeybee pathogen *Melisoccocus plutonius*. We have studied the mechanisms that drive the evolution of a highly dynamic genomic region of about 30 kb that contains genes for biofilm formation and family 70 glycosyl hydrolases (GH70), which are among the most highly abundant extracellular proteins in this bacterium. Moreover, we have found that one antibiotic-producing strain segregated into colonies with different characteristics when grown in the presence of sucrose. One such colony type displayed a mucoid morphology and became unable to inhibit the growth of *M. plutonius* despite retaining the kunkecin plasmid. A comparative study showed that the genomes of the mucoid colonies contained a 14-nucleotide deletion in a chromosomal gene for a response regulator receiver domain of a transcription factor. Our analysis of transcriptomics and proteomics data showed that the expression levels of kunkecin A biosynthesis genes were lower while the expression levels of biofilm-related and nucleotide biosynthesis genes were higher in the mucoid colonies. This suggests that the identified transcription factor serves as an activator of genes for kunkecin biosynthesis and as a repressor of genes for biofilm formation. We will discuss the implications of these results for the ecology of honeybees and their defensive symbionts.

B072

A Bayesian method for bacterial GWAS: genotype-phenotype association & prediction

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Abstract

Genome-wide association studies (GWAS) provide an exciting opportunity to study the relation between genotypes and a phenotype of interest, but applying current GWAS methods to bacterial data has critical limitations. In particular, the quantity of genomic data available has exploded over recent years, rendering computational efficiency essential for large-scale analyses; in addition, one widely reported measure of antimicrobial resistance (AMR), minimum inhibitory concentrations (MICs), is often treated as continuous. I discuss my development of a Bayesian GWAS model that aims to ameliorate some of these limitations, including the use of lineage clustering as a scalable method to correct for population structure-related effects, and offering continuous, ordinal categorical, and logistic regression to facilitate more accurate analysis of different AMR phenotypes. In addition to identifying variants strongly associated with the phenotype, the fitted model also offers phenotype prediction from query genotypes and heritability estimation. Relatedly, I describe the use of linkage disequilibrium pruning to attempt to reduce the size of the genomic datasets needed to identify these correlations in bacterial GWAS with little loss of information. These tools help fill the need for efficient, accurate and user-friendly bacterial GWAS methods.

B074

ESKAPE Room: A Genetic Circuit for Beta-Lactamase Detection in ESKAPE Bacteria.

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Abstract

This study introduces a novel genetic circuit designed for the targeted detection of beta-lactamase-producing ESKAPE bacteria, pivotal contributors to antimicrobial resistance (AMR). The circuit employs an AmpR promoter initiating the expression of a TetR repressor gene, chosen for their strategic incorporation. This repressor gene, following the AND gate genetic logic, suppresses eGFP marker expression under the PR1promoter and TetO operator to which the TetR binds. In the absence of AMR microbes, the repressor remains active, ensuring the marker's silence. However, in the presence of ESKAPE bacteria with beta-lactamase activity, the repressor is deactivated, allowing the expression of the marker and indicating the presence of AMR microbes. This AND gate genetic logic provides specificity in the detection process. This computationally constructed genetic circuit finds future applications in diverse settings, including real-time monitoring of AMR in environmental samples, rapid screening of biological specimens, and early detection in clinical settings. Utilizing a microfluidic device enhances the sensitivity of the detection process, suitable for point-of-care diagnostics and continuous AMR monitoring. The circuit's adaptability is enhanced by the choice of repressor genes and the option between a protein-based fluorescent marker or a quantum dot. The ESKAPE Room genetic circuit represents an innovative and attractive approach, utilizing specific genetic logic for efficient beta-lactamase detection in ESKAPE bacteria across various applications.

B075

Genomic diversity of non-typhoidal Salmonella found within patients suffering from gastroenteritis in Norfolk, UK

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Abstract

In the UK, non-typhoidal *Salmonella* (NTS) pose a significant threat to public health, causing widespread foodborne illness and outbreaks. For surveillance and outbreak investigations the UK Health Security Agency's Gastrointestinal Bacterial Reference Unit utilizes Single Nucleotide Polymorphism (SNP) analysis. A 5-SNP threshold is crucial as isolates within this range likely share a common infection source. However, standard microbiological practice typically involves analysing a single isolated colony per sample, raising the question of how representative a single colony might be of the corresponding infection.

This study focused upon within-patient diversity of NTS causing gastroenteritis by isolating and sequencing up to 20 isolates per stool sample from eight patients. Hybrid DNA sequencing using Illumina NextSeq and Oxford Nanopore MinION was employed to construct closed genomes. Bioinformatics methods were used to analyse serotypes, antimicrobial resistance (AMR) profiles, genome structures, and SNP profiles.

Our results showed that, despite identical serotypes within patient samples, diversity emerged at the SNP level, accompanied by variations in antibiotic resistance and cryptic plasmid profiles. Notably, SNP distances larger than the conventional 5-SNP threshold were common, suggesting the need to reconsider this stringent criterion. Relaxing the threshold could enhance outbreak linkage and identify additional infection sources. The study also highlighted the risk of relying on a single colony for pathogen characterization, as evidenced by observed discrepancies in AMR profiles.

B076

The Genomic Epidemiology of Antimicrobial Resistance in a Global Collection of Escherichia coli isolates

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Abstract

Antimicrobial resistance (AMR) is an existential threat with profound individual and economic global burden. Escherichia coli is a Gram-negative bacillus that is a leading cause of AMR infection-associated mortality; however, the breadth of its resistome and its link with species evolution is still unclear. This study aimed to characterise the genomic epidemiology of AMR within a large (n=875) collection of clinical E. coli isolates from a global surveillance study. These isolates were randomly submitted from 59 diagnostic laboratories across 35 countries and then short-read sequenced on an Illumina platform. We retrieved the raw sequences from the European Nucleotide Archive. Following sequence assembly and filtering for completeness, contamination and taxonomy, 745 of 875 assemblies remained. We characterised their AMR gene (ARG) repertoire in detail, classifying them by gene family, drug class and mechanism using the CARD database. Most ARGs were present in larger contigs (p <2.2x10-16) and several ARGs co-occurred in the same contig. All 745 isolates had at least 1 ARG with an average of 60 per sequence. The most prevalent ARGs (acrB, acrA, and kpnF) were linked to efflux pumps. These genes were present in 99.7% (793/795) of isolates, highlighting a widespread potential to resist multiple antibiotic classes. The least prevalent ARGs were aminoglycoside acetyltransferases AAC(3)-VIa, AAC(6')-Ib-cr1, and AAC(6')-Ib-cr4, each present in one isolate. Finally, by using pangenomic and phylogenomic analyses, we illustrate the extent of AMR genetic diversity within E. coli genomes and frame their resistome in an evolutionary context.

B077

Uncovering strain-specific genetic factors within Lactobacillus jensenii isolated from preterm and full-term pregnancies

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Abstract

Each year, 15 million infants are born preterm (<37 weeks gestation), representing the leading cause of mortality for children under the age of five. Whilst there is no single cause, factors such as maternal genetics, environmental interactions, and the vaginal microbiome have been associated with an increased risk of preterm birth. Previous studies show that a vaginal microbiota dominated by Lactobacillus is, in contrast to communities containing a mixture of genera, associated with full-term birth. However, this binary principle does not fully consider more nuanced interactions between bacterial strains and the host. Here, through a combination of analyses involving genome-sequenced isolates and strain-resolved metagenomics, we identify a subgroup of L. jensenii strains from preterm pregnancies that are phylogenetically distinct from strains from full-term pregnancies. Detailed analysis reveals several genetic signatures that distinguish preterm birth strains, including genes predicted to be involved in cell wall synthesis, and lactate and acetate metabolism. Notably, we identify a distinct gene cluster involved in cell surface protein synthesis in our preterm strains. This study contributes to the ongoing search for molecular biomarkers linked to preterm birth and opens up new avenues for exploring strain-level variations and mechanisms that may contribute to preterm birth.

B078

Integration of transcription factor binding sites in Vibrio cholerae.

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Abstract

Cyclic-di-GMP is a ubiquitous bacterial secondary messenger. The human pathogen *Vibrio* cholerae provides an interesting model to investigate Cyclic-di-GMP signalling, given its ability to transition between two distinct environmental niches, the aquatic ecosystem, and the human intestinal tract. In *V. cholerae* cyclic-di-GMP signalling modulates the changes between motile and sessile lifeforms. Cyclic-di-GMP signals can be transduced by the global transcription factor VpsR. In this work we have mapped the genome-wide distribution of VpsR in *V. cholerae*. In total, VpsR targets 54 loci, including upstream of genes involved in biofilm formation, response regulators and virulence. We found that many VpsR binding sites overlap with potential cAMP receptor protein (CRP) targets. CRP transduces cAMP signalling in response to carbon starvation. This overlap occurs because of a high sequence similarity between VpsR and CRP binding site consensus sequences. An overlapping binding site was identified within the vpsR promoter element. At this overlapping binding site co-binding was observed which resulted in transcription being downregulated. This is an example of how cyclic-di-GMP and cyclic-AMP signalling can be integrated into the genome to control gene expression in response to changing environmental stimuli. Significantly, since the vpsR promoter is regulated by the integration of cyclic-di-GMP and cyclic-AMP signals this will have a downstream impact on the gene expression of biofilm formation, response regulators and virulence factors identified in the VpsR regulon.

B079

PorB in *Neisseria meningitidis*: Investigating links to hyperinvasive clonal complexes

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Abstract

The oropharyngeal commensal bacterium *Neisseria meningitidis* can cause several diseases in humans including meningitis, septicaemia, meningococcal STIs, conjunctivitis, and pneumonia. The landscape of invasive meningococcal disease (IMD) is in a perpetual epidemiological flux at least in part because most genetic diversity is observed within carriage isolates rather than IMD, with outbreaks caused by a few 'hyperinvasive lineages' recognised as members of clonal complexes (ccs). Despite the use of several successful vaccines, meningococcal disease remains a threat.

Protein-based, broad spectrum vaccine targets for *N. meningitidis* often include PorB a major outer membrane protein (OMP). In addition, PorB plays a role in host-pathogen interactions by influencing the severity of the immune response to infection. Within *N. meningitidis,* there are two classes of PorB, class 2 and class 3. Three hyperinvasive ccs associated with class 2 PorB are cc11, cc23, and cc8. On the other hand, cc32, cc41/44, and c269 are predominantly associated with class 3 PorB.

We investigated the association of PorB variants, and specifically the presence of class 2 or class 3 PorB variants with clonal complex. A Neighbour Joining phylogeny of the *porB* gene was reconstructed with data from 28,128 isolates, including 1,844 unique alleles. A minimum spanning tree of 25,604 isolates was also drawn using core genome MLST (cgMLST), showing strong between clonal complex and PorB class.

B080

Exploring plasmids carrying antimicrobial resistance genes in the hospital sink drain microbiome

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Abstract

Hospital sink drains contain a complex microbial ecosystem termed the hospital sink drain microbiome, which acts as a reservoir for antimicrobial resistance genes (ARGs). Many of these ARGs are carried on mobile genetic elements such as plasmids. The hospital sink drain acts as a prime location for plasmid transmission and therefore could be a major contributor to ARG transfer among opportunistic pathogens, but is often overlooked in favour of sewage wastewater or human-associated microbiomes such as those in the gut. To gain insight into microbial ecology and patterns and drivers of gene exchange in this habitat, we swabbed and sampled P-trap water from 18 hospital sinks. From these samples, we performed shotgun metagenomic sequencing as well as collecting and characterising a range of isolates for whole-genome sequencing using long-read technology. Our particular focus is on *Pseudomonas* species — ubiquitous opportunistic pathogens known to spread via plumbing. On-going analysis aims to use the shotgun metagenomic data to reveal the diversity across the sink drains sampled, and the resistome of each sink. From the isolates, analysis aims to identify plasmids and assess the features and diversity of the plasmids across different species in the sink drain samples. Overall, these initial results characterise the resistome and plasmidome of real-world hospital sink drains.

B081

Genome evolution of antimicrobial resistance in staphylococci

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Abstract

Staphylococci are common commensal bacteria in human and animal epidermal microbiomes but can cause serious infections requiring antimicrobial chemotherapy. The emergence of antimicrobial resistance (AMR) is a major problem, and methicillin-resistant S. aureus (MRSA) are a significant global health challenge. Mobile genetic elements are key to the spread of AMR, including the staphylococcal cassette chromosome mec (SCCmec) that houses the mecA resistance gene. However, little is known about the forces that led to the emergence of specific MRSA lineages and the forces that maintain them in diverse populations. Here we use new bioinformatics tools to identify genes linked to the horizontal acquisition of SCCmec. Accounting for linkage disequilibrium, the masked genome-wide association study (mGWAS) analyses of ~1000 S. aureus isolates identified genes and alleles that covaried with SCCmec gene presence and absence. Integrated phylogenetic analyses catalogues the chronology of potentiating genome adaptation providing new insights into the genetics of antimicrobial resistance in staphylococci. By understanding the origin of resistance, the recipient strains, and how large-scale change is accommodated in the genome, it may be possible to limit activities that promote further AMR evolution.

B082

Developing a mathematical model to predict how vaccines shape the population genetics of *Streptococcus pneumoniae*

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Abstract

Streptococcus pneumoniae is a pathogen that populates the human nasal cavity and the respiratory tract. While many healthy humans carry it asymptomatically, *S. pneumoniae* can turn pathogenic and cause severe diseases, including pneumonia and sepsis. The burden of disease is especially high in young children and in low-income countries. A number of vaccines have been developed, all of which target only those serotypes that cause disease most frequently, while most of the over 100 *S. pneumoniae* serotypes are not affected by the vaccines. Vaccines therefore decrease the frequency of vaccine types but not overall carriage. Many non-vaccine types benefit from the introduction of the vaccines and increase in frequency. It is crucial to predict which non-vaccine serotypes will benefit the most from the introduction of a vaccine to avoid a surge in prevalence of especially pathogenic strains. Based on an existing model, I am developing a stochastic compartmental model that predicts how the composition of the bacterial communities of *S. pneumoniae* change after the introduction of vaccines. With my model, it is computationally efficient to simulate populations with large numbers of individuals and fit the model to different data sets. Therefore, my model can be used to simulate the effect of existing and theoretically possible vaccines and answer an array of questions. Currently, we are investigating whether whole genome sequences are essential for predicting the population development or whether serotype data, which are easier and less costly to acquire and therefore more accessible to low-income countries, hold sufficient information.

B083

Surveillance of Travel-associated Isolates Elucidates the Diversity of Non-pandemic *Vibrio cholerae*

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Abstract

Cholera disease - characterised by acute watery diarrhoea and severe dehydration - is caused by the Gram-negative bacterium *Vibrio cholerae*. Cholera presents a significant global health burden, with an estimated 1.3–5 million cases and 21,000–143,000 deaths annually. The seventh, ongoing pandemic is caused by toxigenic strains of the O1 El Tor biotype (7PET). However, isolates from non-7PET lineages are also able to cause sporadic disease with less defined virulence mechanisms and often milder symptoms. Thus, the burden of non-7PET disease may be underestimated. Here we show the potential importance of surveillance through travel-associated isolates in capturing a more complete picture of the total diversity of *V. cholerae* in a country. Thirty-four *V. cholerae* isolates were obtained from travellers returning from Indonesia to Australia between 2005 and 2017. These were whole genome sequenced, placed in phylogenetic context, and screened for genetic elements of interest, revealing that 30 isolates fell within diverse non-7PET lineages and four within the 7PET lineage. Both 7PET and non-7PET isolates harboured genetic determinants for antimicrobial resistance and virulence. This study demonstrates how sentinel travel surveillance can enrich the knowledge of *V. cholerae* diversity in countries where routine sequencing is not performed, and highlights the disease-causing potential of non-toxigenic, non-7PET lineages. As non-7PET *V. cholerae* is associated with sporadic disease of lower severity than 7PET *V. cholerae*, these findings also suggest the ease of carrying these infections across long distances, and thus the role of travel in long-range carriage of cholera.

B084

100 years of *Bordetella pertussis* evolution in the UK

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Abstract

The evolution of *Bordetella pertussis* from its closest ancestors involved the expansion of the copy number of insertion sequence element IS481. Intra-genomic recombination between copies of IS481 has caused extensive genome degradation and structural rearrangements. The first vaccine, a whole-cell vaccine (WCV), for prevention of pertussis disease, also known as whooping cough, was first added to the childhood vaccination programme in the UK in 1951, with the acellular vaccine (ACV) replacing it in 2001. Recent research has suggested that vaccine-mediated selection may be causing waning immunity seen in response the ACV. However, this research has lacked isolates from the pre-vaccine and WCV eras preventing the full trajectory of recent *B. pertussis* evolution to be observed. Here we present a comparison of the whole genome assemblies of 200 isolates covering a 100-year period in the UK. These isolates cover the pre-vaccine, WCV and ACV eras to evidence how further genome degradation, structural arrangements, SNP mutations and change in allele types of vaccine antigens correlate with a response to vaccine-mediated selection pressure. The balance of gene gain and loss is further explored with a pangenome analysis of an additional 924 publicly available closed genomes using new tool ggCaller. This gives finer resolution on 'regions of differences' previously described in the species, and how these differences correlate to ACV and WCV usage. These regions of difference allude to the genes important in vaccine escape for *B. pertussis*, which may be an important contributor to declining immunity in countries with ACV use.

B085

How do interactions between mobile genetic elements enhance resistance gene spread?

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Abstract

Horizontal gene transfer (HGT) is a core driver of rapid bacterial evolution and plasmids are key to this process, spreading adaptive traits with ecological and clinical importance, such as resistance genes. It is increasingly clear that plasmid-borne resistance genes are usually 'nested' on transposons, enabling mobilisation and carriage by plasmids. This web of interactions (plasmid-transposon-chromosome) is predicted to have important implications for the spread of resistance genes. Using a laboratory microcosm system and computer modelling, we investigated how different plasmid 'vehicles' affected the spread of a chromosomal, transposon-borne resistance gene and how the transposability of a trait can affect plasmid population dynamics. We found that resistance gene mobilisation varied across a panel of plasmids largely independent of conjugation rate, suggesting that other plasmid features, such as gene content, may influence chromosomal gene mobilisation. To test the contribution of intra-genome gene mobility to the persistence and spread of traits, we constructed plasmids carrying mobile (i.e. on a transposon) and non-mobile (i.e. integrated in the plasmid backbone) resistance genes, and tested the effects on MGE persistence under varying environmental selection regimes. To more broadly explore how plasmid features (conjugation rate, maintenance cost) combined with transposon features (transposition rate) affect resistance gene spread, we developed an agent-based model to disentangle the complexities of these interactions and predict how trait spread is affected in a variety of environmental scenarios. Understanding the nested hierarchies of MGEs and consequent gene exchange has important application in predicting the evolution of traits like antimicrobial resistance in microbial communities.

B086

The impact of ZnO supplementation on piglet gut microbiome and the resistome using shotgun metagenomics.

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Abstract

ZnO supplementation of weaner diet reduces post-weaning diarrhoea, thereby improving piglet growth and welfare. However, from June 2024 pharmacological ZnO supplementation of pig feed will be prohibited in the UK, due to evidence that Zinc co-selects for antimicrobial resistance within the gut microbiota. We collected pen faecal samples from weaners on two commercial farms to examine the effect of ZnO removal from feed on the microbiome. One herd (A) raises outdoor-bred piglets, weaned to straw yards, the second herd (B) raises indoor-bred piglets, weaned indoors on slats. Both farms ran trials with parallel groups fed diet with/without ZnO supplementation, at a level of 1250ppm (Farm A) and 2500ppm (Farm B). Ten pen pool samples were taken from each group 10-14 days post-wean, metagenomic DNA extracted and DNA sequenced using the Illumina shotgun metagenomic method.

We used KMA to identify reads mapped to antibiotic resistance genes from the Resfinder database, metal resistance genes from the MEGAres database, and a curated panel of relevant virulence genes. Taxonomic classification was conducted using Kraken2. A differential abundance analysis was performed using ALDex2 to pinpoint genes significantly enriched in samples with/ without ZnO treatment. The shotgun metagenomic data revealed that ZnO-negative samples from Farm A exhibited a higher prevalence of particular resistance and virulence genes, a higher abundance of *Enterobacteriaceae* and an overall higher microbiome diversity. The implications of this microbiome signal on the emergence of resistance as well as the production and well-being of pigs when ZnO is removed warrant further investigation.

B087

Seeing beyond proteins in bacterial genomes – Development and application of computational methods for finding conserved and functional genomic regions outside protein-coding annotations in *Mycobacterium tuberculosis*.

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Abstract

Genome annotation has traditionally focused on regions that are translated into proteins (CDS regions) but non-coding elements in *Mycobacterium tuberculosis* (Mtb), and related pathogenic mycobacteria in general, are believed to play an important role in enabling these species to adapt to specific hosts, environmental and lifestyle challenges. Sequence conservation at distinct evolutionary timeframes (within species, phylum, genus) can help identify elements in the genome that may have a functional role, guiding experiments to the more promising genomic regions. However, determining whether a region is coding or non-coding is not a trivial task, especially in the light of studies discovering as yet unannotated small open reading frames in the Mtb genome. In this study: a) We use ancestral mutational patterns reconstructed from Mtb strains to build a phylogenetically-aware test that distinguishes coding from non-coding regions. b) We apply a relative entropy measure to assess conservation of sequence regions upstream and downstream of homologous CDS within two reference datasets: one comprising ~200 Mycobacteriaceae and one comprising ~1700 Actinobacteria. c) We measure the extent of covariation of base pairing in regions surrounding CDS in Actinobacteria to identify sequences that could have a functional, non-coding role that relies on secondary structure. Our results support some of the non-coding elements discussed in the literature and additionally suggest novel regions for further investigation.

B088

Unexpected ExPEC: *Escherichia coli* sequence type 744 phylogenetic, genomic and phenotypic characterization

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Abstract

Background: *Escherichia coli* sequence type (ST) 744 represent a zoonotic high-risk international lineage capable of causing extraintestinal infections. We recovered strains of ST744 within multiple of our projects focused on antimicrobial resistance monitoring, therefore we decided to examine its global cohort.

Materials/methods: We performed a phylogenetic analysis of 915 strains (32 from our collection and 883 from EnteroBase), screened the genomes for relevant genomic markers, and performed comparative genomics with MG1655 using two long-read sequenced strains. We phenotypically characterized 32 strains in optimal and stress-inducing conditions, to explore the effect of prominent ST744-associated stress-coping and metabolism-related genes, using growth curves and quantitative fluorescence microscopy. For these assays, our panel of 32 strains was compared with MG1655 and other Extra-Intestinal Pathogenic *E. coli* (ExPEC) strains.

Results: *E. coli* ST744 phylogeny revealed two major clusters linked to the dominant serotype (H9:O101 and H10:O101). Despite largely lacking the typical ExPEC virulence-associated genes, 170 strains were assigned as clinically relevant (e.g., isolated from blood, urine). On the other hand, 96% of the strains carried antibiotic resistance genes (ARGs) to three or more antibiotic classes with ten ARGs on average. Interestingly, several livestock-associated clades lacked plasmid replicons, yet still possessed multiple ARGs in a genomic region with an *intI1* integrase. Preliminary results of ongoing phenotypic characterization indicated differences in response to osmotic stress.

Conclusions: Besides antibiotic resistance, ST744 showed atypical characteristics for ExPEC. Focus on phenotypic features and stress response in ExPEC can help cover the gap in understanding the emergence and success of ExPEC.

B089

Mobilization of Anti-phage Systems via Lateral Transduction

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Abstract

Bacteriophages (phages) are viruses of bacteria which exert significant evolutionary pressure on bacterial populations, driving the development of diverse anti-phage defence systems by bacteria. These systems are characterized by their dynamic nature, being rapidly acquired or lost from bacterial genomes, which allow bacteria to adapt to new threats. However, the mechanisms underlying the mobility of anti-phage systems remain unexplored. Lateral transduction (LT) is a process of horizontal gene transfer where large sections of bacterial DNA can be transferred between bacteria by phages. Here, we show that LT is a major factor in the spread of anti-phage systems. To test this hypothesis, we conducted experiments on two representative bacterial species: *Staphylococcus aureus* and *Escherichia coli*. Indeed, we observed highly efficient sharing of anti-phage systems in these model systems. In *S. aureus*, we saw the efficient transduction of defence islands, including key anti-phage and virulence elements. Similarly, in *E. coli*, chromosomal hotspots related to anti-phage defence were successfully mobilized. These results not only confirm the high transduction efficiency of LT but also demonstrate its role in enhancing phage resistance in recipient strains. Since LT is a common mechanism of horizontal gene transfer, we anticipate that LT is a key driver of the spread of anti-phage systems in bacteria, with profound implications for our understanding of bacterial evolution and survival strategies in the face of phage predation.

B090

Longitudinal genomic surveillance studies reveal the structures and dynamics of an intensive care unit *Acinetobacter baumannii* population

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Abstract

Acinetobacter baumannii is a nosocomial pathogen of global concern and an urgent priority for novel antimicrobials. Therapeutic options for carbapenem-resistant *A. baumannii* (CRAB) are severely limited, so infection prevention and control (IPC) measures must play a role in limiting its clinical impact. Genomic surveillance can inform IPC strategies and improve our understanding of CRAB persistence, transmission, and evolution in hospitals.

We conducted two 13-week longitudinal genomic surveillance studies of *A. baumannii* in a 28-bed intensive care unit (ICU) in Hangzhou, China. The first was conducted in August-October 2019 and the second in May-July 2021, following delays associated with the COVID-19 pandemic. Informed by the first study, a bundled set of targeted IPC interventions was implemented for eight months prior to and throughout the second study. In total, 1,070 *A. baumannii* isolates from patients (223), clinical specimens (32), and the ICU environment (815) were whole-genome sequenced.

The complex ICU population was shaped by patient-associated introductions and environmental persistence of phylogenetically-distinct CRAB clusters. Across the studies, we observed multiple CRAB clusters appearing in recently-admitted patient screening samples before spreading to different bed unit or room environments and subsequently being acquired by previously CRAB-negative patients.

Fewer CRAB were isolated in 2021, but phenotypic carbapenem resistance levels increased due to a major population shift. Clusters of GC2, a globally-disseminated OXA-23 carbapenemase-producing lineage, dominated the population in 2019 (99.5%). In 2021, GC2 clusters (50.8% of CRAB) appeared to be in competition with ST164 (49.2%), a newly-emerged and highly carbapenem-resistant clone that co-produced OXA-23 and NDM-1.

B091

Global genomic diversity of *Pseudomonas aeruginosa* in bronchiectasis

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Abstract

Pseudomonas aeruginosa is the dominant pathogen causing lung infections in people with both cystic fibrosis (CF) and bronchiectasis, associated with worsened disease outcomes. Unlike CF, existing research of *P. aeruginosa* infections in bronchiectasis is limited. There is an urgent need for better understanding of the genomic diversity of *P. aeruginosa* infections in people with bronchiectasis to facilitate the development of effective treatment regimens, particularly in the context of rising rates of antimicrobial resistance worldwide. We performed whole genome sequencing of 2,854 *P. aeruginosa* isolates from 180 patients with bronchiectasis from Europe, Australia, South Africa, the USA, Canada, and Asia, the largest sequenced collection of *P. aeruginosa* isolates from bronchiectasis to date. We observed high genetic diversity between infections, with 77% of sequence types isolated from a single patient and low incidence of epidemic strains (7% of patients). We provide evidence for the mutational targets driving *P. aeruginosa* evolution during chronic infection in the bronchiectasis lung, some similarly observed in CF (e.g., biofilm and iron acquisition) and some distinct (e.g., pyocin production and a novel efflux pump). We also show a high incidence of antimicrobial resistance-associated mutations and acquired resistance genes, in particular multidrug efflux and fluoroquinolone resistance mechanisms. Our findings suggest that while some similarities exist with *P. aeruginosa* infections in CF, notable distinct features of bronchiectasis infections require more targeted studies to guide development of improved treatment strategies.

B092

Metagenomic investigation into the use of alternatives to antimicrobials on optimisation of gut health and performance of broiler chickens

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Abstract

Understanding the relationship between chicken gut health and gut microbiota is vital in the optimisation of bird performance and welfare. Historically, antimicrobials have been used in the industry to prevent diseases and promote growth; However, increasing demand on raising broilers without the use of antibiotics, concerns over the development of antimicrobial resistance, and gaps in vaccination have prompted research into use of alternatives to antimicrobials (ATAs) and other supplementary approaches for the maintenance of gut health.

A longitudinal study involving three broiler production cycles (C0, C1, C2) was conducted in a commercial farm to compare five control strategies involving ATAs (T2 – T6), versus the use of ionophore coccidiostat *salinomycin* as control (T1). Several zootechnical performance and health parameters were monitored weekly. Caecal samples at clearing were collected from C1 and C2 and processed for Illumina short-read shotgun sequencing. Bioinformatic analysis using the *phyloFlash* pipeline was subsequently performed.

Initial statistical analysis of performance data showed that both within and between flock variation is present. ATA treatment groups were associated with significant increase in feed conversion ratio measures during several points of production. Microbial alpha diversity metrics of treatments were similar, but T1 and T5 demonstrated significantly higher fisher alpha and species richness. Analysis of beta diversity metrics also revealed significant differences between the groups. Differential analysis demonstrates varied microbial community signatures per treatment group. According to our preliminary results, alternative approaches impact both broiler performance and microbial diversity. Further investigation is yet to be conducted to better understand these differences.

B093

A novel method to remove environmental contamination from ancient oral metagenomic samples

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Abstract

When studying ancient DNA, contamination is one of the foremost concerns. Contaminant DNA from the environment, the laboratory, and the researchers themselves are pervasive in ancient oral microbiome samples. Dental calculus is a commonly studied material when investigating ancient oral microbiomes and there are several methods commonly employed to decontaminate dental calculus itself in the lab as well as the resulting aDNA sequences via computational methods. As ancient oral microbiome research is relatively new, there is not currently a universal standard for decontamination processes. Additionally, due to the nature of archaeological work such as this, it is not always possible to access the material (e.g. matching bone sample) needed to complete environmental decontamination during computational analysis.

To address these issues, we have developed a method to decontaminate aDNA sequences derived from dental calculus when environmental contaminant control samples are not available. This method works by combining lists of bacterial taxa common among several environmental contaminant control samples to create a new, composite control list. The composite control list is then used to decontaminate calculus samples by filtering out OUTs that are common between the calculus samples and the list. Our results show that such master lists of contaminant species has the possibility to improve aDNA metagenomic processing and cleaner taxonomic presence patterns in samples.

B095

Plasmid sharing between cephalosporin-resistant human gut microbiome and disease-associated *Enterobacteriaceae* in Vietnamese community

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Abstract

Plasmids in human gut microbiome are the major reservoirs contributing to the global spread of antibiotic resistance (AMR) genes via frequent horizontal gene transfer (HGT) events, particularly in *Enterobacteriaceae*. In Vietnam, considerable efforts have been made to understand the spread of extended spectrum beta lactamase (ESBL)-encoding genes under One Health approach. However, these efforts generally lacked a thorough investigation of the broader ESBL plasmid transmission patterns between ecological niches. To study this, we first isolated and characterised the genetic structure of cephalosporin-resistant conjugative plasmids from human gut microbiome. We then investigated the extent of sharing between major plasmid groups in human gut microbiome with putative plasmid sequences obtained from human disease-associated pathogens (*Shigella sonnei*, non-typhoidal *Salmonella*, extra-intestinal pathogenic *Escherichia coli* ST131) and pig *E. coli*; all collected between 2010 and 2018 within Vietnam. IncI1, IncB/O/K/Z and IncF are found to be the most common plasmid groups, carrying mainly $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$ and $bla_{CTX\text{-}M\text{-}55}$, in both human-derived commensal bacteria and pathogens in Vietnam. Plasmid phylogeny and network analysis reveal the commonality of plasmid sharing between human disease-associated pathogens and human gut microbiome, and, to a much lesser extent, animal gut microbiome. Notably, epidemiological evidence and conjugation assays suggested that HGT dictates the degree of plasmid spread between bacterial species, while the extent of its spread is determined by bacterial clonal expansion. These results highlight the importance of human gut microbiome as the reservoirs for ESBL plasmids in clinically important gut-associated pathogens in Vietnam and should be targeted for future interventions.

B096

How to lose a plasmid in ten days – understanding plasmid-host compatibility to predict their spread and maintenance in bacterial communities

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Abstract

The remarkable ability of bacteria to rapidly acquire resistance to antibiotics is a major challenge for healthcare professionals and the pharmaceutical industry, and mobile genetic elements such as plasmids are the key agents spreading AMR within and amongst bacterial communities. However, our limited understanding of plasmid – bacteria dynamics hampers our ability to predict plasmid maintenance and dissemination to pathogens and to develop therapeutic treatments to efficiently tackle AMR. To investigate the varying effects of plasmid acquisition across different bacteria, we transferred members of a widespread emerging multi-drug resistance megaplasmid family to >40 diverse strains of the opportunistic pathogen Pseudomonas aeruginosa and assessed the effects on bacterial fitness. We show that the same plasmid can impose varying fitness costs on strains of the same species, and, in some cases, provides fitness benefits even in the absence of antibiotics. The phenotypic effects of megaplasmid acquisition were correlated with genome sequences to identify underlying molecular mechanisms. Our findings suggest that specific genetic and molecular factors dictate a plasmid's behaviour within its host, and that therapeutic strategies designed against one combination of pathogen-AMR plasmid might not apply universally, particularly in the context of multi-species microbiomes. Our findings suggest a need to look beyond existing notions of AMR plasmids persisting in the presence of antibiotics and focus on plasmid-host compatibility that promote or prevent plasmid persistence. Ultimately, we will be able to predict microbiomes in which transmission of AMR plasmids are likely to be heightened, allowing us to better control the spread of antimicrobial resistance.

B098

A Genome-Wide Association Study (GWAS) of *Neisseria meningitidis*: Investigating the Invasive Meningococcal Phenotype.

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Abstract

Neisseria meningitidis, whilst often a common commensal of the human respiratory tract, is also a global leading cause of bacterial meningitis and septicaemia, making it an important threat to public health. Whilst previous work has identified meningococcal factors that facilitate the transition from carriage to pathogenicity, the polygenic nature of the invasive N. meningitidis phenotype is yet to be fully elucidated. We employed a genome-wide association study (GWAS) approach, implementing two separate software tools on our dataset, TreeWAS and Pyseer, to identify and characterise systematic genetic differences between carriage and disease N. meningitidis isolates. Our dataset consisted of 2428 N. meningitidis isolates (1008 disease, and 1420 carriage isolates) obtained from the PubMLST open-access database, providing statistical power and integrating two effective approaches, with the aim of identifying new genetic variants associated with the invasive meningococcal phenotype. Characterising the bacterial genetic factors contributing to IMD will facilitate genome-based predictions about the likely clinical and epidemiological outcomes of differing meningococcal variants, enabling improved public health and clinical interventions.

B099

Repeated exposure to lethal acidic stress drives evolution of acid sensitive Listeria monocytogenes strains towards becoming more acid resistant.

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Abstract

Listeria monocytogenes is a gram-positive food-borne pathogen that causes life-threatening infection. Acid resistance is important for the pathogenic lifestyle of this bacterium because it must survive transit of acidic stomach environment prior to establishing an infection in the ileum. However, acid resistance is a highly variable phenotype, even among clinical isolates. Truncations of a recently identified regulatory gene of acid resistance, *gadR*, strongly associate with acid sensitive phenotypes but do not fully explain strain-to-strain differences. In this study, we sought to investigate the acid sensitive nature of reference strain EGD-e (*gadR-*) and clinical isolates MQ140025 (*gadR+*) and G-026 (*gadR+*). Cultures of these strains were repeatedly exposed to lethal acidic challenge (pH 3 or pH 2.5) and subsequently recovered in BHI (~20h). The cultures of all three strains displayed enhanced acid resistance over only four challenge-recovery cycles (i.e. 4 days). All acid resistant derivatives (ARD) of EGD-e reverted the non-sense mutation in *gadR* and gained GadR-mediated GAD activity. While none of MQ140025-ARD or G-026-ARD displayed increase in GAD activity except G-026-ARD3. Not surprisingly, G-026-ARD3 also gained greater acid resistance than other G-026-ARDs. Taken together, we show that acid sensitive *L. monocytogenes* readily acquire resistance and therefore the risk associated with acid sensitive isolates should not be overlooked. Moreover, despite the critical role that GadR plays in acid resistance in *L. monocytogenes*, there are other mechanisms contributing to this phenotypical trait that remain to be uncovered. These results present novel challenges in controlling and managing food safety risks associated with *L. monocytogenes*.

B100

Genetic tuning and evolutionary origin of the internal stop in *prfB* of *Pseudomonas fluorescens* SBW25

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Abstract

Translation termination is the last step in bacterial translation and involves the recognition of stop codons (UAA, UGA, UAG) by release factors (RFs). The efficiency of stop codons varies considerably, depending on the identity of the stop codon, genomic context, and physiological conditions. While most stop codons are fairly efficient, the *prfB* gene - encoding RF2, which recognizes UGA stop codons - carries an inefficient stop codon within its coding sequence. During translation, the recognition of the internal stop by RF2 (leading to severely truncated RF2) competes with a +1 ribosomal frameshift event (leading to full-length RF2). Since the balance of these two alternatives depends on RF2 concentration, the internal stop acts as an elegant autoregulation system.

In this study, we investigate the evolutionary origins of the internal stop codon of the *prfB* gene and the subsequent tuning of termination efficiency. In particular: (i) can the efficiency of the *prfB* internal stop be manipulated, and (ii) what are the molecular and evolutionary consequences?

So far, we have used genetic engineering to make changes to the efficiency of the *prfB* internal stop in the model bacterium *Pseudomonas fluorescens* SBW25. We have quantified the effects of these changes on bacterial growth, and are investigating how the deleterious changes can be compensated through a serial transfer evolution experiment.

To summarize, the study investigates how the efficiency of stop codon tuned and inefficient stop codons are utilized in *prfB* gene by experimental and computational approaches.

B101

Gene regulatory rewiring to re-evolve a lost swimming phenotype is largely conserved across the *Pseudomonas* clade but hampered in some nutrient environments

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Abstract

Gene regulatory network architecture tends to be conserved across species groups. By making the same catastrophic regulatory deletion in several different species of *Pseudomonas* we can examine the impact of genetic background on the rewiring of a broken network.

Here we look at five different Pseudomonads: *Pseudomonas putida* KT2440, *Pseudomonas ogarae* F113, *Pseudomonas syringae* pv. *tomato* DC3000, and *Pseudomonas fluorescens* strains Pf0-1 and SBW25.

These bacteria are immotile due to disruption of the flagellar master regulator FleQ. They are selected to restore swimming motility through starvation on 0.25% agar plates, which they do after just a few days, but the routes taken to restore this phenotype and the outcome of this differ between them. We screen them for mutations that have restored the swimming phenotype. All lines restore a swimming phenotype through rewiring of the nitrogen master regulator NtrC, suggesting conservation of this evolutionary path across the *Pseudomonas* clade. However, for some lines, restoration of swimming is dependent on the nutrient environment in which they evolved. While *P. ogarae* F113 and *P. syringae* pv. *tomato* DC3000 will readily restore swimming in the minimal media M9, we rarely, if ever, see a swimming phenotype evolve in a complex LB environment, highlighting the important, but sometimes understated, role environment can play in evolution.

B102

CRISPR-Cas antimicrobials eliminate epidemic animal-associated IncI1 resistance plasmids from Salmonella and E. coli

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Abstract

Antimicrobial resistance (AMR) is a significant public health challenge, attributable to over one million deaths per annum. Despite the fundamental role of antibiotics in the clinic, around 75% of manufactured antibiotics are designated for use in animals. Thus, AMR is harboured in the microflora of food-producing animals, frequently on mobile elements that could transfer resistance into pathogens. We utilise CRISPR as an alternative to antibiotics, and as a tool that can specifically eliminate AMR plasmids from the population. As a proof-of-concept we targeted the epidemic IncI1 resistance plasmids with the type I-E CRISPR system from *Escherichia coli*. A guide spacer sequence designed against the $bla_{\text{CTX-M-1}}$ gene was cloned into CRISPR-Cas plasmid vectors and then mobilized by conjugation into wild type *E. coli* recipient strains as well as *Salmonella* Typhimurium strain LT2. These contained the IncI1 resistance plasmids pESBL-138 and pPE13096, and retention of these plasmids was assessed by plating onto media containing the antibiotic cefotaxime. Plasmid curing efficiency was determined by comparison with a negative control CRISPR plasmid that contained a non-targeting spacer. In *E. coli*, pESBL-138 and pPE13096 carriage were each reduced by 100-fold, whereas in *Salmonella* plasmid carriage was reduced by 1000-fold, without significant differences between plasmids. In both cases, loss of resistance plasmid resulted in the death of the recipient strain, rather than the intended plasmid curing effect, likely due to toxin-antitoxin systems on the plasmids. This work provides the impetus for use of CRISPR-Cas in AMR remediation in agricultural contexts through conjugative delivery.

B103

A rapid, simple and cost-effective workflow for the preparation of bacterial samples for sequencing

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Abstract

It is estimated that hospital associated infections (HAI) cost $35 to $45 billion in the USA and the NHS £3 billion in the UK, as well as countless lives. Implementation of whole genome sequencing (WGS) could allow the early detection and direct the intervention of outbreaks. This could result in a huge reduction in spending and save many lives. The key to effective implementation of counter-measures is the speed from sample collection to result. Therefore, any method that offers a timesaving is of particular interest. Further to this, any method that offers a cost saving could aid the widespread implementation of WGS. In this work we propose a method for the rapid preparation of cultured bacterial samples for Sanger and Next Generation sequencing. Utilisation of Microzone's "Free From" technology allows for a fast, efficient workflow only requiring a centrifugation step and doesn't require automation.

B104

Toward High-Throughput Engineering of Undomesticated Microbes.

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Abstract

Most research involving genetic engineering is restricted to well-studied organisms such as Escherichia coli and Bacillus subtilis, but the methodology for their engineering does not translate easily to other microorganisms. There is currently a lack of understanding and genetic tools available for non-model lab strains. Though large collections of microbes are now being isolated by high-throughput culturomics protocols, the ability to study or manipulate these collections through genetic engineering represents a major bottleneck. In this work, we aim to democratize genetic engineering by developing a high throughput pipeline to identify the engineerability of cultured microbial community members at large. Sixteen vectors were constructed by golden gate cloning using the BEVA (Bacteria Expression Vector Archive) architecture, containing one of four origins of replication, along with one of four antibiotic resistance genes (Geddes et al., 2019)*. Broad host range origins were selected to facilitate replication in a wide range of bacterial families. Members of the vector library were tagged with a green fluorescent protein and a distinct nucleotide barcode and stored in the E. coli donor strain WM3064. We explored a variety of approaches including FACS (Fluorescence-activated Cell Sorting) and Illumina next-gen sequencing of molecular barcodes to develop a high-throughput pipeline for assessing the engineerability of a wide range of cultured microbes en mass. Overall, we believe these approaches can enable rapid toolkit development for efficient engineering of most cultivatable target microorganisms.

*Geddes, Barney A., et al. "A bacterial expression Vector archive (BEVA) for Flexible Modular Assembly of Golden Gate-compatible vectors." Frontiers in Microbiology, vol. 9, 2019, https://doi.org/10.3389/fmicb.2018.03345.

B105

Updating microbial genetics tools for their application to the human gut microbiome

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Abstract

The human gut microbiome consists of trillions of organisms from all three domains of life. This complex and interconnected system is driven by the biological interactions between its constituents (host-microbe, microbe-microbe) and plays a major role in human health and development. However, we currently know very little about the specific mechanisms driving these interactions. The application of microbial genetics techniques to the gut microbiome presents a powerful opportunity to tease out the functional basis for community dynamics, yet the majority of gut-associated microbes are genetically intractable and resistant to genetic manipulation. This is primarily due to an incompatibility between traditional genetic tools (that were developed for model organisms like *E. coli*) and the phylogenetically diverse non-model organisms that are associated with the human gut. In response, we have developed a DNA transfer strategy that has been optimized for compatibility with diverse gut organisms and the anaerobic conditions in which they are cultured. This strategy incorporates microbial defence system inactivation, a kill-switch for counter-selection following DNA transfer, and a conjugation system that can be “primed” for diverse recipients. This represents the first microbiome-specific genetic tool in this emerging era of microbiome genetics and engineering and will potentially enable a deeper and more mechanistic understanding of human gut microbiome interactions.

B106

Chromosomal cleaning of mobile genetic elements via lateral transduction in Staphylococcus aureus

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Abstract

Genetic transduction emerges as a crucial driving force in bacterial evolution, playing a pivotal role in adaptation. Among its various modes, lateral transduction (LT) stands out as exceptionally potent, enabling the transfer of bacterial DNA at frequencies surpassing any known transduction mechanism by at least 1,000 times. However, the necessity for such a remarkable transfer of chromosomal DNA remains poorly understood. In this context, we propose that LT significantly contributes to bacterial chromosomal cleansing in Staphylococcus aureus. Our results demonstrate that through LT, parasitic mobile genetic elements can be effectively removed from the chromosome. This process alleviates the potential costs associated with carrying these elements in bacterial populations. This study thus offers valuable insights into the role of LT in bacterial evolution, shedding light on its implications for the emergence of novel clinical strains.

B107

Global diversity and evolution of *Salmonella* Panama, an understudied serovar causing gastrointestinal and invasive disease worldwide

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Abstract

Nontyphoidal *Salmonella* (NTS) has emerged as one of the pathogens most frequently isolated from the human bloodstream, with only a small number of serovars able to colonise normally sterile sites to cause invasive NTS (iNTS) disease. *S.* Panama is responsible for a significant number of cases of iNTS disease. However, this serovar has not been investigated in detail despite global dissemination, numerous outbreaks, and a reported association with iNTS disease.

We analysed a collection of 836 *S.* Panama isolates from all continents obtained between 1931 and 2019. Using combined epidemiological and whole genome sequencing data we determined the population structure, evolutionary history, and inferred geo-temporal dissemination.

This first large-scale phylogenetic analysis of *S.* Panama revealed multiple geographically linked clades, and regional trends in antimicrobial resistance profiles. Multidrug resistant isolates belonged to two phylogenetic clades circulating in Europe and Asia/Oceania, whereas most isolates were pan-susceptible to antibiotics and belonged to clades circulating in the American continent. The multidrug resistant isolates also showed the highest invasiveness indices based on the conservation of 196 extra-intestinal predictor genes.

Our findings provide an important baseline for understanding *S.* Panama infection in the future and suggest the need for monitoring of multidrug resistant clades with an elevated invasiveness index by ongoing surveillance as such clades may pose an increased public health risk.

B108

Phage mediated maintenance of bacterial defence system diversity

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Abstract

Prokaryotes have evolved a diverse arsenal of defence systems to protect them from parasites such as bacteriophages, with the most prolific being restriction modification (RM) systems. RM systems function by methylating self-DNA at a specific sequence, and cleaving DNA if an unmethylated copy of the sequence is recognised. However, bacteriophages can escape these systems by chance acquisition of the methylation signature upon infection, allowing all progeny to infect cells with the same RM system. Being readily overcome, it is unclear why the remarkable RM system diversity observed in nature is maintained.

In communities, as bacteriophages can only escape the RM systems of one strain at a time, selection should favour escaping the dominant population's RM systems, due to higher host availability and chance of initial escape. The infected population will then decline, while populations with other RM systems increase, until selection favours switching to infect a newly dominant strain. This negative frequency dependence could explain why high RM system diversity is maintained.

We test this hypothesis by evolving lytic bacteriophage, lambda *vir*, on *E. coli* strains with different RM systems and monitoring cell and phage population dynamics. Our experiments provide evidence of the predicted negative frequency dependence of RM systems. Additionally, we show that even when one system is easier for the phage to escape it is still not lost due to these fluctuating infection dynamics. Here we provide novel evidence that bacteriophages maintain RM system diversity, and thus potentially also contribute to the maintenance of species diversity.

B109

Limited Impact of Intergenic Region Variation on Gene Expression Among Phylogenetically-Related *Neisseria meningitidis* Isolates

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Abstract

Neisseria meningitidis is a significant cause of bacterial meningitis, particularly affecting children and immunocompromised individuals. Previous studies have shown that variation in noncoding regions plays a role in the expression of specific genes within *N. meningitidis*, often contributing to virulence, but to date no work has investigated associations of intergenic variation with expression across the entire genome. A 2009 study at the University of Nottingham has produced a large collection of whole-genome sequenced *N. meningitidis* isolates from both carriage and disease groups. Combined with the increasing affordability of RNA sequencing, this has provided the opportunity to investigate expression differences amongst the whole genomes of related isolates. The transcriptomes of eight serogroup Y, clonal complex 23 carriage isolates taken from five individuals in the University of Nottingham study have been sequenced and intergenic variation data for the isolates collected from the PubMLST database. Linear regression on the data identifies 126 loci with a significant correlation between expression changes and intergenic variation, including 32 loci involved in gene regulation or the production of outer membrane proteins. Clusters of Orthologous Genes (COG) enrichment analysis shows a disproportionate number of several COG categories within significantly differentially expressed genes compared to the entire dataset, including the categories for transcription, defense mechanisms and energy production and conversion. Together, these results show that intergenic variation plays a role in the expression of gene regulators, potentially contributing to expression changes in hundreds of regulated genes.

B110

The effect of Restriction-Modification systems against plasmid conjugation

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Abstract

In bacteria, genes conferring antibiotic resistance are mostly carried on conjugative plasmids, mobile elements which spread horizontally among bacterial hosts. Bacteria carry immune systems which defend them against genetic parasites, but how effective they are against plasmid conjugation is poorly understood. In particular, it is unknown to what extend the most prevalent bacterial immune systems, restriction-modification (RM) systems, act as a barrier against plasmids in the wild, and whether this can be predicted from host and/or plasmid genetics.

Here, we measured how effective RM systems are against a collection of natural resistance plasmids in *Escherichia coli*. We uncovered variation in defense efficiency ranging from none to 10^5-fold protection. We then explored the contribution of RM type, plasmid escape, and anti-RM genes to this variation. As expected, we find that restriction efficiency overall depends on the number of recognition sites present on the plasmids. Some plasmids significantly avoid recognition sites for specific RM systems, and are only weakly restricted by these systems. However, this effect of recognition site number is absent for most type I restriction systems and for the type II EcoRII, suggesting that other plasmid-encoded functions play a role. We then show experimentally that both plasmid-encoded and host-encoded cytosine methylases can fully protect plasmids against EcoRII restriction. Finally, we show that other recently discovered anti-restriction and recombination genes provide protection to plasmids against type I RM systems.

Overall, our results show that plasmids have evolved varied strategies which limit RM efficacy as a barrier to plasmid transfer by conjugation.

B111

Plasmids maintain low mobility to prevent eradication in nature

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Abstract

Plasmids have classically been considered as highly-mobile contributors to horizontal gene transfer in bacteria, readily transferring antimicrobial resistance/virulence factors that compromise treatment outcomes. However, the transfer efficiency of plasmids by phage transduction – one important mechanism of plasmid mobility – is markedly lower than that of other mobile genetic elements, and the evolutionary basis for this remains unclear.

Here, we show that plasmids have low mobility because they lack phage packaging sites, although the addition of these increased transfer efficiency with no impact on plasmid fitness. Moreover, we demonstrate high plasmid transduction can be easily evolved in the laboratory through serial passaging in the presence of a phage. Sequencing revealed the mechanism of increased transferability to be incorporation of phage DNA into plasmids. Crucially, there was no negative evolutionary effect of highly-transducible plasmids in terms of their stability/burden on bacterial cells, suggesting an alternative reason why natural plasmids restrict their transfer efficiency.

We propose that in mixed bacterial communities, the sharing of "common goods" encoded on plasmids can provide protection from threats to the whole population even in the absence of plasmid mobility, and experimentally prove this using anti-phage systems and antibiotic resistance genes carried by plasmids. Furthermore, we highlight that this community-level protection works optimally when plasmids are present in populations at equal proportions, and is compromised when a single dominant plasmid takes over the community. Thus, we provide an ecological scenario to explain the benefits of low plasmid mobility, showing reduced transfer is key to preventing plasmid eradication.

B112

cyPhyRNA-seq as a Method to Identify tRNA Targets of Yeast Anticodon Nucleases

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Abstract

Killer plasmids are linear, cytosolic, double-stranded DNA molecules found in several species of yeast. Killer plasmid-containing yeasts secrete toxins called anticodon nucleases (ACNases), which destroy specific tRNAs in other yeasts, killing them. We hypothesised that ACNase toxins could be responsible for the unusual genetic codes used in some yeast species, where the codon CUG is read by a $tRNA^{Ser}_{CAG}$ or a $tRNA^{Ala}_{CAG}$ instead of $tRNA^{Leu}_{CAG}$. However, only 4 ACNase toxins are known, sharing very low (~30%) amino acid identity due to rapid evolution, and none of the known ACNases targets $tRNA^{Leu}_{CAG}$. Killer plasmids are rare and often missing from genome sequence assemblies. After making a pipeline to automatically download and assemble raw Illumina data from every publicly available budding yeast species in the NCBI Sequence Repository Archive, we identified **27 new candidate toxin genes.**

To identify the tRNAs targeted by these new toxins, we used a technique called cyPhyRNA-seq. After cleaving their target tRNA, ACNases leave a 2’,3’-cyclic phosphate (instead of a 3’-OH) on the 5’-fragment and a 5’-OH (instead of 5’-P) at the start of the 3’-fragment. In cyPhyRNA-seq, an adapter is specifically ligated to RNA molecules ending with 2’3’-cyclic phosphates. Ligation products are converted to cDNA, ligated to a second adapter, and submitted to high-throughput sequencing. We characterised the targets of a new toxin of interest from *Lachancea kluyveri* (LkT), and a known toxin from *Pichae acaciae* (PaT). cyPhy-RNAseq correctly identified the primary and secondary targets of PaT, $tRNA^{Gln}_{UUG}$ and $tRNA^{Gln}_{CUG}$, and identified two potential targets for LkT.

B113

Evolutionary arms race between a yeast homing genetic element and its genomic target.

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Abstract

We are investigating WHO elements, a new type of homing genetic element that was recently discovered by our lab in the yeast genus Torulaspora. WHO elements are related to, but different from, inteins and homing introns, two major classes of well-known homing elements. WHO elements are about 4 kb long. They encode endonucleases that cut genomic DNA at one particular target site, in the glycolytic gene FBA1. The double-strand DNA break in FBA1 is then repaired by integrating a WHO element. This process allows WHO elements to integrate into previously “empty” alleles of FBA1, causing them to spread through the population. WHO elements and their target site in FBA1 are both highly variable in sequence, and they appear to be locked into an evolutionary arms race with each other. We hypothesise that in this arms race, WHO endonucleases are under selection to cleave as many alleles (sequence variants) of FBA1 as possible, while FBA1 alleles are under selection to avoid being cleaved. To test this hypothesis, we are using experimental assays to determine the sensitivity or resistance of different FBA1 alleles to cleavage by WHO endonucleases from different families. We are also mutation-scanning the target site of one WHO endonuclease in order to determine its precise sequence requirements for cleaving FBA1. Our goal is to test the arms race hypothesis, and to determine how these families of endonucleases and cleavage sites are co-evolving.

B114

Beyond the genome: linking genotype to phenotype for West African *Salmonella* Enteritidis

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Abstract

Salmonella enterica is a major causative agent of gastroenteritis in humans, with serovars *S*. Enteritidis and *S*. Typhimurium being the most common. Over recent decades these serovars have become a leading cause of bloodstream infection, particularly in immunocompromised individuals across sub-Saharan Africa. The high case fatality-rate of 14.5% underscores the urgent need for comprehensive studies on pathovariants such as *S*. Typhimurium sequence type (ST)313. Nevertheless, it is noteworthy that around one-third of all human invasive non-Typhoidal Salmonella (iNTS) cases arise from *S*. Enteritidis, rather than *S*. Typhimurium. Despite this significant prevalence, *S*. Enteritidis remains a serovar that has not received sufficient research attention.

This research focusses on identifying the genetic properties that distinguish *Salmonella* isolates capable of causing bloodstream infections from those causing gastroenteritis, with a particular emphasis on the Central-Eastern and West African clades. Notably, West African countries bear a disproportionately high burden of these infections. We are conducting a comparative analysis between the West African clade and the Global Epidemic clade to identify specific genetic determinants that facilitate host adaptation and contribute to enhanced virulence. Using a random barcode transposon site sequencing (RB-TnSeq) approach, we are leveraging the power of genetic techniques to systematically interrogate iNTS genomes at a functional level. Using a combination of *in vitro* and intramacrophage experiments, we have identified candidate virulence genes This method allows for the identification of crucial genes and mechanisms that underpin the increased virulence and adaptability of iNTS, providing valuable insights for future targeted intervention strategies.

B115

Convergent evolution of *Streptococcus pyogenes* isolates towards acapsular/high-toxin expression increasing prevalence

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Abstract

Streptococcus pyogenes (also known as group A Streptococcus or StrepA) causes a range of human infections from skin or throat infections to life-threatening invasive disease. In 2022-2023 there was an unprecedented early seasonal upsurge in S. pyogenes disease, during which we collected 341 throat and skin isolates identified during routine NHS clinical microbiology diagnostics at the Northern General Hospital, Sheffield. We carried out whole genome sequencing and analysis for diversity and pathogenicity factors.

The hyaluronic capsule has long been considered essential for S. pyogenes adhesion and virulence, however we have recently found lineages of S. pyogenes that increased in prevalence having lost the ability to produce capsule through recombination or inactivating mutations. In addition, these lineages have gained increased NADase and Streptolysin O (SLO) toxin expression through recombination. Within our throat isolates from the 2022-23 upsurge, 41% lacked the ability to produce capsule and were also predicted to highly express NADase and SLO, compared to just 2% which were encapsulated with low toxin expression. Overall, 90% of throat isolates showed high toxin expression. The opposite was observed within skin isolates, of which just 22% lacked capsule and highly expressed toxin compared to 44% being encapsulated with low toxin expression.
Some emm-types have recently changed, with 2022-23 emm75 losing the ability to produce capsule, compared to those collected in recent previous years. These results suggest an association between capsule, toxin expression and tissue tropism and highlights a key role for toxin expression in the pathogenesis of streptococcal throat infections compared to skin infections.

B116

Exploring the Evolutionary Dynamics of Plasmid Genomes Across the Prokaryotic Tree of Life

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Abstract

Plasmids are self-replicating genetic elements that exist alongside chromosomal DNA in prokaryotes. They are known for their role in the dissemination of genes critical to virulence and antibiotic resistance. However, the rules governing the structure and dynamics of their genomes remain largely unknown; plasmid evolution differs significantly from that of chromosomal DNA. Of special interest is large-scale genetic movement between different plasmid backbones. Here, we studied a collection of over 40,000 plasmid sequences across 3,006 species to understand their dynamics of gene sharing. An efficient alignment-free approach was implemented to calculate pairwise similarity and subsequently cluster the plasmids into 13,845 independent plasmid types. Within these clusters, 1,419 plasmid groups contain plasmid members who were not all discovered within the same species. Through comparative analysis, we identified a wide spectrum of genetic sharing among plasmid groups. While different plasmid types share little similarity as measured by Jaccard Index, some plasmid types demonstrate containment within other plasmid types. These complex 'fusion' plasmids are currently being explored as to which plasmids are being combined. Among the most shared regions, we detected several clusters of antibiotic resistance genes highly conserved across multiple plasmid types, which highlights their ability to disseminate through diverse plasmid backbones. Likewise, various genes controlling plasmid replication, historically used for plasmid typing, were highly shared between plasmid clusters, indicating convergence in replication strategies and their poor predictability of plasmid identity. Altogether, this study shows that classifying and understanding genetic interactions between plasmids may highlight the biological rules underlying their large-scale evolution.

B117

Identifying phase variable genes from simple sequence repeats within different phylogeographical populations of *Helicobacter pylori*.

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Abstract

Helicobacter pylori was established in the human stomach 100,000 years ago, prior to the first modern human migration out of Africa approximately 60,000 years ago. *H. pylori* presently infects approximately half of the global population and is associated with an increased risk of gastric diseases, including cancer. Phase variation through simple sequence repeats (SSRs) can alter the expression of a gene/s reversibly, stochastically and with high frequency in a population. Phase variation driven phenotypic diversity can lead to the long term survival of the population when encountering changing selection pressures. This study aims to identify phase variable genes within different phylogeographical strains of *H. pylori* and consider the functions genes are associated with. A total of 36 reference isolates belonging to the following phylogeographical sub-populations were used: hpEurope (9), hspWAfrica (6), hpAfrica2 (2), hspSouthIndia (2), hspEAsia (8), hspAmerind (9). Whole genome sequences from NCBI were annotated (Prokka) before analysis with PhasomeIT, a tool for identifying SSRs within a genome and linking them to open reading frames. There appear to be no differences in the total number of phase variable genes across each phylogeographical sub-population. Considering repeat tract lengths from 5 - 29 bp, 91.2 % of these come from single nucleotide repeats of thymine (T) and 8.5% of these come from single nucleotide repeats of guanine (G). Similar to *Campylobacter* and *Neisseria*, *H. pylori* repeat tracts were identified as belonging to cell surface associated proteins. Genes encoding proteins for the flagella were also among those identified as containing SSRs.

B119

Using co-occurrence analysis to determine gene interactions in short-read genomic data

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Abstract

Short-read sequencing makes *de novo* assembly difficult at the site of repeated sequences, which cannot be bridged if the reads are shorter than the length of the repeat. The inability to traverse repetitive regions This results in numerous contigs, often terminating at mobile genetic element (MGE) sites. Long-read technology can overcome these limitations by spanning repetitive regions and resolve MGEs, but is expensive and the majority of reads on genetic databases are Illumina short reads. We need novel techniques to infer gene genetic context, in particular co-location on MGEs from short read data which can be used to target isolates for long read sequencing.

Here, we present a probabilistic analysis of gene co-occurrence to address this. We assembled 772 genomes of *Escherichia coli* and *Klebsiella pneumoniae* previously sequenced and isolated from blood and stool samples from Malawi, to understand the genetic location of antimicrobial resistance (AMR) genes relative to each other. This analysis employed a probabilistic model to identify significant co-occurrences, revealing genetic interactions among AMR genes.

Noteworthy connections included *aac(6')-Ib-cr* - bla_{OXA-1} - *catB3* and its associations with $bla_{CTX-M-15}$ and bla_{TEM-1B}. To confirm our findings, we sequenced 10 *E. coli* isolates with long-read sequencing and compared inferred connections from co-occurrence analysis with the actual genomic environment, the results showed that the *aac(6')-Ib-cr* -bla_{OXA-1} -*catB3* feature was always located together and flanked by two IS*26*.

In the future, co-occurrence analysis could be used to inform treatment regimens by highlighting co-occurring resistance, thereby optimizing antibiotic use.

B120

Characterisation of novel weberviruses and examination of their depolymerases

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Abstract

Members of the genus *Webervirus* are lytic bacteriophages that infect *Klebsiella* spp. and exhibit depolymerase activity on their host. The first described member of this genus, *Webervirus KP36*, was isolated from a Polish wastewater plant and there are currently 32 species of *Webervirus* listed within the International Committee on Taxonomy of Viruses database. Depolymerases are bacteriophage proteins that degrade the bacterial capsule, allowing for the virus to infect the cell and causing susceptibility to the innate immune response. Seven novel bacteriophages were isolated from sewage samples before being sequenced, imaged, and characterised. This characterisation involved annotation with PROKKA using the PHROGs hidden Markov model, and phylogenetic analysis using ViPTree, which showed that the novel bacteriophages belonged to the genus *Webervirus*. PhageClouds was used to identify phages related to weberviruses in metagenome-assembled genome (MAG) datasets. This identified 60 MAGs that were used to create a database of webervirus genomes in combination with 59 genomes comprising GenBank and the novel genomes. These genomes were also checked for quality, leaving 107 high-quality genomes for use in further analyses. Depolymerases were identified in these genomes using BLASTp vs a curated database of depolymerase sequences. This search identified 143 depolymerases, with each phage genome encoding at least one. Phylogenetic analysis grouped these sequences into four distinct clusters. Synthetic biology and gene expression work is being carried out to examine the activity of these theoretical depolymerases to determine if their activity can be predicted based on which cluster they belong to.

B121

Strain-resolved metagenomic using protal

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Abstract

Introduction: Metagenomic profilers process sequencing data to reveal the composition of microbial communities in a multitude of different environments. Even though profiling microbial communities on species-level is a routine analysis, contemporary metagenomic profilers often suffer from common shortcomings such as inflated diversity, high computational demands, differing taxonomic systems and out-of-date reference databases. For strain-level metagenomics the situation is even more complicated, as there are fewer tools that are also computationally demanding and mostly tailored towards specific use-cases.

Result: Here, we present_ protal (profiling through alignment), a newly developed metagenomic profiler. Protal offers accurate species-level profiles surpassing all contemporary profilers, while also resolving microbial strains. Protal is highly efficient, being at least 5x faster than comparable tools. Further, protal's full use and support of the GTDB database results in a more than twofold increase in species representation in metagenomes.

Conclusion: protal offers superior taxonomic sensitivity and precision and strain-level resolution at faster speeds than current software, democratizing the analysis of metagenomes.

B125

The Influence of Lateral Transduction on Bacterial Genome Content and Structure

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Abstract

Background. Lateral transduction (LT) may be the most powerful form of horizontal gene transfer yet discovered. During LT, large fragments of the bacterial chromosome are packaged and transferred by phages, via delayed prophage excision. *Staphylococcus aureus*, a major human and animal pathogen, has a strongly conserved genome structure but diverse accessory genome. The localisation of phage attachment (*attB*) sites could enable around 60% of the *S. aureus* chromosome to undergo LT-related mobilisation, facilitating gene gain and loss and/or genome maintenance.

Objective. To investigate the influence of LT on the genome content and structure of *S. aureus*, using an array of comparative genomic approaches.

Methods. A dereplicated dataset of 200 complete *S. aureus* genomes was analysed. Based on the distribution of phage integration sites and direction of packaging, the genome was differentiated into two regions: R1 which is predicted to be subject to LT, and R2 which is predicted to be unaffected by LT. Tools such as Gubbins, Pseudofinder and digIS were used to compare the frequency and location of recombination hotspots, pseudogenes, insertion sequences and essential genes in these two regions.

Results & Conclusions. Recombination hotspots occur downstream of *attB* sites, corresponding to the phage packaging orientations. Significant differences were observed between the R1 and R2 regions: pseudogenes and insertion sequences were more densely distributed in R2, while the majority of essential genes were more frequently located in the *attB*-abundant region R1. Overall, these data are consistent with LT demonstrating a key influence on *S. aureus* genome organisation and diversity.

B126

Secretion systems in the Vibrionaceae family

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Abstract

Vibrionaceae is a bacterial family with hundreds of species that inhabit a variety of mostly marine environments. The most well studied member is *Vibrio cholerae*, a pathogen which causes watery diarrhoea in humans. The family also contains other pathogens that cause gastroenteritis in humans such as *Vibrio vulnificus* and *Vibrio parahaemolyticus*. I curated a dataset of publicly available Vibrionaceae genomes from GenBank to help understand how these phylogenetically distant species have evolved to become human pathogens that cause diarrhoea. Using comparative genomics, I show that all three species have different secretion systems linked to cytotoxicity. *V.vulnificus* and *V.cholerae* have the rtx toxin transporter system. *V.parahaemolyticus* have two different type 3 secretion system gene (T3SS) while *V.cholerae* only has T3SS2. V.cholerae have a type 6 secretion (T6SS) as well. The same systems are found in other species closely related to *V.cholerae*; *V.mimicus* and *V.paracholerae*. Like *V.cholerae* they both have the T3SS2, a T6SS and rtx transporter system, although they lack the rtxA toxin gene. *V.mimicus* is also the only species other than *V.cholerae* that has the two cholera toxin subunits and the tcp pilus genes, except for tcpA. These analyses have shown that whilst all three species may cause diarrhoea in humans they have different systems that may help facilitate that. It also highlights that *V.mimicus* has all the same secretion systems as *V.cholerae* and requires further interrogation of its genome content to determine its status as an important emerging pathogen.

B127

Genome-wide association studies of *Haemophilus influenzae* identify multiple loci and genetic variants associated with invasive diseases and capsule acquisition

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Abstract

Haemophilus influenzae resides in the human nasopharynx as commensal but can cross the epithelial barrier, causing invasive diseases such as meningitis and septicaemia. This Gram-negative bacterium is classified based on the expression of capsular polysaccharides. Although capsule expression was known as the primary determinant of invasiveness, epidemiological reports in later years showed that encapsulated and unencapsulated (or nontypeable) *H. influenzae* (NTHi) are equally likely to cause invasive diseases. Therefore, our study aims to identify loci in the accessory genome and genetic variants in the pangenome of *H. influenzae* associated with invasive disease and capsule acquisition. We performed pangenome analysis and genome-wide association studies (GWAS) on 2,555 high-quality *H. influenzae* draft genomes. After masking the capsule region and accounting for population structure, we found that the presence/absence of genes in the accessory genome could predict whether an isolate caused disease, particularly meningitis, with an R^2 of 0.24 to 0.44. 25% of accessory genes associated with disease were involved in the metabolism pathway, and 20% of these had a function in iron transport (*fhuA/B/C* and *TonB* dependent receptor) and iron storage proteins (*ftnA/B*). K-mers-based GWAS revealed variants in *murF* gene, the product of which was participated in cell wall biosynthesis, were significantly associated with *H. influenzae* invasive disease. Interestingly, one of the top hits associated with capsule expression was a gene encoding for *H. influenzae* bacteriocin, pyocin. These genes whose products were exposed to external environment were thought to be under high selective pressure and, therefore could drive the evolutionary trajectory of *H. influenzae*.

B128

Genomic investigation of colonising *Staphylococcus pseudintermedius* in healthy humans

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Abstract

Staphylococcus pseudintermedius is part of the canine microbiota and a common cause of skin and ear infections in dogs. Human infections have predominantly been reported in immunocompromised individuals, alongside asymptomatic detection in veterinary staff. Human colonisation with *S. pseudintermedius* has not been comprehensively studied due to limited sample sizes. Utilising the extensive CARRIAGE dataset including of nasal swabs of over 20,000 healthy human participants across England, human and canine *S. pseudintermedius* strains.

We isolated 70 strains of *S. pseudintermedius*, from 53 participants and performed whole genome sequencing. To understand the population structure, we conducted *de novo* assembly of these isolates and incorporated publicly available sequences (canines 92%, 6% human). This dataset was used for phylogenetic reconstruction and comparative analysis.

Isolation of *S. pseudintermedius* was higher in participants reporting dog ownership (90.5% 48/53), compared to the overall cohort (41.4% pet ownership (n=6678)). Notably, S. *pseudintermedius* was isolated longitudinally from seven participants. Phylogenetic reconstruction revealed a highly diverse S. *pseudintermedius* population in humans, mirroring the diversity in canine isolates. Isolates from each participant were closely related, consistent with in-host persistence. These findings suggest that transmission of *S. pseudintermedius* to humans is not unusual and may potentially lead to persistence over time. Two methicillin resistant isolates were identified, implying a role in antimicrobial resistance evolution, emphasising the significance of *S. pseudintermedius* in the context of One-Health.

These findings highlight *S. pseudintermedius* transmission from dogs to humans, prompting further exploration of zoonotic potential, antimicrobial resistance and associated risk factors.

B129

Understanding the role of genetic background in determining the evolutionary outcome of gene regulatory network rewiring events

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Abstract

Previous work in the Taylor lab, using motility rescue as a model, has shown rapid and repeatable rewiring between the nitrogen and flagellar networks in *Pseudomonas fluorescens*. In the absence of the flagellar master regulator (FleQ), NtrC (response regulator associated with nitrogen uptake) is co-opted to resurrect motility. My research aims to explore the role of global regulatory network architecture, and the wider genome, in determining gene regulatory rewiring pathways. Initial work explores whether flagellar motility rescue can be repeatably achieved in other gram-negative bacteria, and the rewiring routes capable of this. We aim to delete the flagellar master regulator across several different bacteria: *Pseudomonas aeruginosa, Caulobacter crescentus, Vibrio cholerae* and *Escherichia coli,* which represent a diverse arrangement of flagellar regulatory networks. Replicate independent lines will be placed under strong selection for motility, and motile isolates sampled, and genome sequenced, to identify rewiring routes. Initial results from *P. aeruginosa* show flagellar motility rapidly evolved, similar to *P. fluorescens*; isolates evolved slow motility after as little as 42 hours. Evolved motile mutants will be genome sequenced to identify the rewiring routes used to regain motility. This work will contribute to a larger aim, which is to identify how differences in gene regulatory architecture determines the predictability of evolved rewiring pathways across different bacteria using motility rescue as a model system.

BLOCK B

Session : Prokaryotic Stress Responses – their diversity and regulation

B130

Functional dissection of the periplasmic domain of RcsD, the phosphotransfer protein of the Esherichia coli Rcs system.

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Abstract

The Gram-negative cell envelope represents a formidable barrier to environmental stressors and numerous antimicrobials. In E. coli, as well as other enterobacteria, the Rcs system (Regulator of capsule synthesis) is an important two-component signalling pathway playing a critical role in maintaining the integrity of the bacterial envelope. One of the peculiarities of the Rcs system is the contribution of several auxiliary proteins to its signalling cascade. Among them, RcsD, an inner membrane 100 kDa protein, transfers the phosphate from the histidine kinase (RcsC) to the cytosolic response regulator (RcsB). The cytosolic part of RcsD is indispensable for signalling as it is responsible for the phosphotransferase function.

However, RcsD harbors a large periplasmic domain (69 kDa) with unexplored role(s). In this work, we aim to gain insights into the role of RcsD periplasmic domain by testing the effect of its deletion and/or substitution on regulation of the Rcs system activation and response to stress. Enhancing our understanding of the mechanism of signalling of the Rcs system is expected to pave the way for novel

antibacterial agent development.

B132

Priming Effect by a Signal Promotes Full Activation of Signal Transduction System that Confers Bacterial Pathogenesis

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Abstract

All living organisms respond to environmental stresses in order to survive. In response to environmental cues, signal transduction systems govern global gene expression via post-translational modification. Under stressful conditions, the two-component system in bacteria regulates gene expression for survival and proliferation. Here, we report that the priming effect of a sensor protein by first signal in two-component signal transduction system fully activates a gene cluster for oxidative stress defense and antimicrobial resistance that enable bacteria survive in the host. The transcriptional regulator PmrA and the sensor PmrB form a two-component system in pathogenic bacterium *Acinetobacter baumannii* are required for survival within the innate immune cells of the host. PmrB detects hydrogen peroxide in the periplasmic space of bacteria, and then actives the PmrA regulator protein by phosphorylation. Activated PmrA stimulates gene expression for iron-sulfur cluster assembly and peroxidase to alleviate bacterial oxidative stress. Histidine residues in PmrB are involved in the detection of hydrogen peroxide by the metal cofactor nickel. Inactivation of histidine residues in PmrB renders is hypersensitive to cellular killing by hydrogen peroxide and innate immune cells. Signal sensing of PmrB by oxidative stress is sufficient for activation of antimicrobial resistance system that fully confers bacteria pathogenesis. Our findings indicate that pathogenic bacteria prepare second stress response by the priming effect of two-component signal transduction system by first stress to survive inside the immune cells of an infected host.

B133

Revealing the variable architecture of PspA/IM30 envelope stress responses in γ-proteobacteria

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Abstract

Envelope stress responses have evolved in bacteria to combat stresses that threaten the integrity of the membrane, often leading to dissipation of membrane potential and growth impairment. The Psp system in *Escherichia coli* is one such stress response which can help restore proton motive force and membrane function. The Psp effector protein, PspA, is a PspA/IM30 family protein that is structurally related to mammalian ESCRT-III proteins and is recruited to its site of action at the membrane by accessory proteins encoded with the *psp* operon. However, the canonical PspA protein is not the only PspA/IM30 family protein found in many bacteria, but in each case the accessory proteins that activate the effector differ, suggesting different physiological functions. Here we use an *in silico* structural approach designed to capture the diversity of PspA/IM30 accessory proteins across the γ-proteobacteria, revealing surprising diversity and presence of novel organisations in important human pathogens. The approach benefits from AlphaFold to build a structural landscape of each system, helping to predict biological functions.

B134

Exploring the impacts of the stringent response on the structure and function of ribosomes in Staphylococcus aureus

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Abstract

The stringent response is conserved across bacterial organisms as a means of survival in response to nutrient deprivation. The response enhances the production of secondary signalling molecules referred to as alarmones, specifically guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp). These alarmones regulate crucial metabolic pathways in the cell, reducing DNA replication, nucleotide synthesis, transcription as well as ribosomal maturation and function. We have previously observed that upon induction of the stringent response in *Staphylococcus aureus*, the formation of 70S ribosomes is inhibited by obstructing the interactions of the GTPases Era, RsgA, RbgA and HflX with ribosomal subunits. These GTPases are conserved throughout prokaryotic organisms and are essential for the maturation of the 30S and 50S ribosomal subunits. (p)ppGpp binds to the GTPases with a higher affinity than GTP, thus preventing association with immature ribosomal subunits and subsequent maturation events. Even though substantial research has been conducted exploring the effects the absence of any of the four GTPases has on the ribosomal structure, the role of these enzymes in ribosomal subunit maturation is still unclear. Here, we explore the impact of the stringent response, and by extension the effects that inhibition of all the GTPases has on *S. aureus* ribosomal structure to elucidate how the structure and function of the ribosome is altered upon induction of the stringent response.

B135

Polymicrobial interactions: Implications for virulence in chronic lung disease

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Abstract

Cystic fibrosis (CF) is a genetic disease primarily impacting the lungs resulting in chronic airway infections. *Pseudomonas aeruginosa* and *Aspergillus fumigatus* commonly co-colonise the CF airway and interactions between these species play a crucial role in disease severity. This study aims to advance our understanding of these interactions focusing on the modulation of *P. aeruginosa* virulence in co-infections.

A. fumigatus was grown in four different growth media: Luria-Bertani (LB), Malt Extract Broth (MEB), Sabouraud's Dextrose Broth (SDB), and Minimal Essential Media+10% Fetal Bovine Serum (MEM+10%FBS) for 72 hours and *P. aeruginosa* (PA01) (OD_{600}:0.6) exposed to the *A. fumigatus* supernatants (AFsn) for 24h at 37°C. The influence of AFsn on biofilm, pyoverdine and pyocyanin production by *P. aeruginosa* was investigated. RNA sequencing of PA01 exposed to *A. fumigatus* was performed on the Illumina NextSeq 2000 with P3 300 cycle paired end.

Biofilm formation by PA01 increased when exposed to AFsn from MEM+10%FBS ($p<0.0001$; 62%) and MEB cultures ($p<0.0001$; 69%). Exposure of PA01 to AFsn resulted in a significant increase in pyoverdine production ($p<0.0001$; 35%[31-101%]) across all media types tested. Pyocyanin production increased when PA01 was exposed to AFsn from MEM+10%FBS ($p<0.0001$; 102%), MEB cultures ($p<0.0001$; 182%) and SDB ($p<0.05$; 10%) cultures. RNA sequencing revealed increased expression of 20 *P. aeruginosa* genes (8 virulence genes) in response to *A. fumigatus* co-culture. Our findings highlight the significant impact of fungal-bacterial interactions in modulating bacterial behaviour in polymicrobial infections.

B136

Making 'antisense' of the PhoPR two-component system in the Mycobacterium tuberculosis complex

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Abstract

Mycobacterium tuberculosis (Mtb) and *Mycobacterium bovis* (Mbovis) have nearly identical genomes but different host specificities and pathogenic profiles. Both lineages demonstrate pervasive antisense transcription which increases in stress conditions, however, comparative transcriptomics reveal differences in the use of transcription start sites and expression of regions outside of protein-coding genes--including antisense RNA (Golby et al., 2007; Malone et al., 2018). We propose that these understudied non-coding elements have an influence on transcription and/or translation in a host-specific manner. Using computational methods with Mtb RNA-seq data to identify and cluster all expressed transcripts in a range of conditions, we have identified an antisense transcript found opposite the *phoR* gene in both Mtb and Mbovis which is highly expressed in acid and stationary growth conditions in Mtb (Stiens et al., 2023). The PhoR sensor-kinase is part of a two-component system that controls the cell response to acid stress by activating the PhoP transcription factor and is active and essential for virulence in both Mtb and Mbovis despite a potentially deleterious SNP in Mbovis *phoR* (Baker et al., 2014; García et al., 2021; Gibson et al., 2022; Gonzalo-Asensio et al., 2014). In order to assess what impact this transcript has on the PhoP regulon, we have made CRISPRi strains in Mtb that knock-down expression of the antisense RNA.

B137

Transcriptomic response of *Pseudomonas aeruginosa* to the CFTR modulator triple therapy Kaftrio / Trikafta.

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Abstract

Pseudomonas aeruginosa is the major pulmonary pathogen of patients with Cystic Fibrosis (CF). CF is the most prevalent autosomal recessive disorder in Caucasians, caused by the inheritance of a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene from both parents. The CFTR protein facilitates the export of chloride ions across epithelial tissues, most notably within the respiratory and digestive organs. CFTR mutations lead to a loss of cilial activity and the accumulation of thick mucous in the lungs which is readily colonised by *P. aeruginosa*. Innovations in drug discovery have led to CFTR modulator drugs becoming available. Three of these drugs, elaxacaftor, tezacaftor and ivacaftor are given as a triple therapy marketed as Kaftrio in the UK and Trikafta in the USA. These drugs stabilise or assist the proper folding of the CFTR protein, leading to significant restoration of transporter function. Several ongoing studies are investigating the impact of Kaftrio on the lung microbiome of CF patients revealing that only around half of patients clear chronic *P. aeruginosa* infections. Whilst Kaftrio specifically targets the human CFTR protein, little is known about its effect on members of the CF lung microbiotia. To address this, we performed RNA-Seq on *P. aeruginosa* PA14 exposed to varying levels of kaftrio or ivacaftor alone. These experiments were performed at 1x and 100x serum concentrations of the drugs as reported by the manufacturer (Vertex Pharmaceuticals, personal communication). Our data shows significant upregulation of iron sequestration genes and components of the osmotic stress response.

B138

The role of selfless viruses in genetic exchange and bacterial evolution

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Abstract

Gene Transfer Agents (GTAs) are genetic elements that facilitate high frequency horizontal gene transfer among diverse prokaryotic organisms under conditions of stress. GTAs are thought to be derived from ancestral bacteriophages that have long since become domesticated by the host. Unlike typical bacteriophages, GTAs do not exhibit any preference for the replication or transfer of their own genes; instead, they exhibit a remarkable capacity to disseminate any cellular DNA to neighbouring cells. I will present data demonstrating how GTA production is regulated by a direct activator protein whose actions are intricately interwoven with multiple stress induced regulatory circuits. I will also present findings about the structure of GTAs and intermediate forms that shed light on the distinctions between GTAs and conventional viruses, such as the ability to indiscriminately package DNA. Crucially, these results provide a framework to prospect genome databases for novel GTAs, to understand their true role in bacterial evolution and to retool them for biotechnology.

B139

Metabolites tune the antimicrobial susceptibility of Mycobacterium tuberculosis

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Abstract

There is an urgent need for new approaches to treat tuberculosis (TB), however antibiotic development is exceptionally challenging due to the remarkable ability of the causative agent *Mycobacterium tuberculosis* to tolerate a variety of host and antimicrobial stressors. An attractive approach is the development of adjuvant therapies that augment the activity of the current antibiotic regime, aiming to not only increase antimicrobial potency but also to reduce the development of antibiotic resistance. Recognizing the key role of bacterial metabolic state on susceptibility to these stressors; our research posits that the direct modulation of cell metabolism, using metabolites from specific pathways, has the potential to improve drug treatment and can serve as a cost-effective approach to develop antibiotic adjuvants. We have identified metabolites that enhance antimicrobial activity and surprisingly also a metabolite that triggers the rapid emergence of isoniazid resistance in *M. tuberculosis*. Exploring these phenotypes using ^{13}C isotopomer analysis we have identified key pathways involved in this phenomenon. This work underscores a sophisticated metabolic strategy to mitigate collateral cellular stress from antibiotic treatment. Informed by these discoveries, we adopted a medicinal chemistry approach to synthesize compounds, allowing us to leverage metabolism as a stressor, and have developed adjuvants that potentiate isoniazid killing of *M. tuberculosis* and counter the development of resistance. Our research furthers the understanding of the bacterial stress response to antibiotic treatment, and provides an innovative approach for development of more effective treatment strategies.

B140

RsuR, from the alpha-proteobacterium *Zymomonas mobilis*, regulates Fe-S cluster biogenesis

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Abstract

Zymomonas mobilis is an alpha-proteobacterium with biotechnological potential due to its small genome and streamlined metabolism. However, little is known about its regulatory networks, including iron-sulfur (Fe-S) cluster biogenesis, a recognized bottleneck in bacterial engineering. *Z. mobilis* is an aerotolerant bacterium that encodes only the *suf* pathway for Fe-S cluster biogenesis. Within this operon is a predicted member of the Rrf-2 family of transcription factors. Many Rrf-2 transcription factors bind Fe-S clusters, which function as a sensor to direct their regulatory activity. In this work, we characterized the Rrf-2 family protein encoded by the *suf* operon, which we designate RsuR (**R**rf-2 ***suf*** **R**egulator). We observed that RsuR, purified in the absence of oxygen, binds an oxygen-sensitive Fe-S cluster. Furthermore, we show that RsuR binds the *suf* promoter around the +1 site, suggesting it occludes RNA polymerase binding. RNAseq analyses indicated that in a Δ*rsuR* mutant, the only transcripts whose expression is increased were those of the *suf* operon, suggesting a high degree of regulatory specificity. The Δ*rsuR* mutant grew more slowly than the wild-type in the presence of oxygen but showed wild-type growth in the absence of oxygen. This response is specifically linked to oxygen availability since we observed no response to other stressors tested (Fe depletion and ROS-generating compounds). Overall, we conclude that RsuR is a highly-specific repressor of the *suf* operon in *Z. mobilis* and that the protein mainly senses oxygen – an activity that is critically dependent on its Fe-S cluster integrity.

B141

Bacterial Impedance Cytometry (BIC) - Gaining insights into bacterial stress responses in individual bacteria exposed to antibiotics.

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Abstract

When bacterial cells are exposed to certain antibiotics (e.g., ciprofloxacin), RecA protein expression is induced causing de-repression of SOS genes and an increase in mutation rate, leading to resistance. We explored whether the Bacterial Impedance Cytometer (BIC) can help to understand changes in electrical properties of bacteria and how these relate to SOS induction in response to antibiotic exposure (Spencer et al., 2020).

E. coli strains were exposed to a dilution series of SOS-inducers, Ciprofloxacin and Nitrofurantoin, at 37°C for 2 hours, ± dequalinium chloride (DEQ), previously described as an inhibitor of general stress response (Zhai et al., 2023). The impedance of individual bacterial cells was measured on the BIC at 5 MHz and 40 MHz frequencies.

The BIC could identify differences in electrical properties of untreated cells compared to those treated at sub-inhibitory concentrations of antibiotics, in a recA positive strain, BW25113, characterised by an increase in electrical diameter of the cell. GFP-reporter strains confirmed the induction of SOS response, measured by upregulation of SOS genes e.g., *recA* and *lexA*. Added dequalinium reduced the observed increase in electrical diameter, consistent with its ability to suppress the general stress response. These changes in electrical properties were not to the same extent in a *recA* mutant strain JW2669, where treated and untreated cells looked similar, confirming that the changes above associated with recA induction.

The BIC is a low volume, rapid technique that may help in studying bacterial stress responses, aiming to find new antibiotics and modulators, to minimise resistance occurrence.

B143

Variability in cell division among anatomical sites shapes Escherichia coli antibiotic survival in a urinary tract infection mouse model

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Abstract

Urinary tract infections (UTI) are a global health problem where *Escherichia coli* is the most common causative agent. Considering the high frequency and recurrent nature of these infections, understanding the factors underlying how *E. coli* escapes antibiotic killing *in vivo* is crucial. Treatment failures in UTI are common and observed even when pathogens are susceptible to antibiotics.

Beside genetic alterations, the microenvironment strongly impact bacterial behavior or survival *in vivo*. Bacterial cell division is of particular interest as slow bacterial growth is associated with survival phenotypes, such as tolerance and persistence in the face of antibiotic therapy. Tolerance and persistence allow bacteria to temporarily survive longer without growing in presence of lethal doses of bactericidal antibiotics.

Using a unique synthetic biology approach that preserves the 'memory' of bacterial behavior *in vivo*, we assessed bacterial division in a UTI mouse model at three sites: the urine, bladder and kidneys. We followed the temporal dynamics of *E. coli* division up to 22 days, in the context of antibiotic therapy. Several *E. coli* strains showed large differences in replicative state between sites, with a greater proportion of dividing bacteria in the kidneys or urine than in the bladder. Bacteria that survived antibiotic treatment were consistently non-dividing in all three sites of infection. This highlights the potential contribution of non-dividing bacteria to recalcitrant infections. Finally, using strains with diverse survival profiles and growth rates *in vitro*, we investigated how cell division and different genetic mechanisms allowing tolerance and persistence impact infection and treatment dynamics.

B144

Exploring the impact of carbon source switching on phenotypic heterogeneity in mycobacteria

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Abstract

Mycobacterium tuberculosis can survive for decades within its human host exposed to continually changing micro-environments and consequently has remarkable metabolic flexibility to adapt to these conditions. A question of potentially therapeutic significance is how nutrient shifts effect phenotypic heterogeneity and tolerance to antibiotics. In this study, we have developed a model system to measure the heterogeneity of M. tuberculosis switching from one carbon source to another using Mycobacterium smegmatis as a containment level 2 surrogate for the pathogen. Using fluorescently labelled reporter strains of M. smegmatis we have shown that carbon source shifts generated subpopulations of differentially growing bacterial cells supportive of the hypothesis that carbon source switching triggers heterogeneity. This work demonstrated that switching from glycolytically (glycerol) to gluconeogenic substrates (pyruvate, lactate or propionate) resulted in a biphasic population of fast growing and slow growing mycobacteria and that this was carbon source dependent. This is highly relevant as this could allow mycobacteria to cope with fluctuating environments and protect the population from adverse effect of stressors such as antibiotics.

B145

Gene Transfer Agents: For the Greater Good?

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Abstract

Gene transfer agents (GTAs) are bacteriophage-like entities which exclusively transfer random fragments of a bacterial genome to surrounding cells via horizontal gene transfer. The transduction-like mechanism utilised by GTAs results in cell lysis of a producing cell and, in turn, sacrifices a subset of the total population. A lack of preference for packaging its own genes for self-replication, as prioritised by traditional transduction, is a key distinction which separates GTAs from bacteriophages. The long-term maintenance of these elements, despite their associated fitness costs, has prompted further questions on the influence GTAs may have on bacterial evolution. Currently, the ecological benefit of GTA production remains largely unknown. To improve our understanding, we performed competition assays in the model GTA producer species, *Rhodobacter capsulatus.* We assessed the performance of the wild type stain and various GTA mutants with different predicted cost-benefit ratios grown under a range of growth conditions and stresses. So far, we have observed that GTA receipt is selectively advantageous under nutrient-rich media and DNA damaging conditions. Furthermore, GTA overproduction increases cell survival in response to the DNA damaging agent zeocin, which has previously been observed in *Caulobacter crescentus*. We also identified two antibiotics, carbenicillin and streptomycin, which induce or repress GTA production in a concentration-dependent manner. Our study makes important steps toward deciphering the function of GTAs and the environmental conditions under which they are beneficial to the host.

B146

Gene Transfer Agents in *Brucella anthropi.*

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Abstract

Gene transfer agents (GTAs) are defective phages that can package and transfer random pieces of the producing cell's genome, but are unable to transfer the genes required for their own production. Previously we showed that a specific GTA activation factor (*gafA*) is essential for production of the model GTA in *Rhodobacter capsulatus* (RcGTA), and this allowed us to understand how multiple host sensor/regulatory systems are integrated to control production of RcGTAs.

Our subsequent aim was to investigate GTA activity in species beyond *Rhodobacter*, with a particular focus on pathogen species that contain *gafA* homologues such as *Brucella* and *Ochrobactrum*. *Brucella* GTA regulation has similarities to the newly discovered *Caulobacter crescentus* GTA where *gafA* is split into two separate genes, *gafY* and *gafZ*, which are in turn controlled by a repressor RogA. Interruption of the *Brucella rogA* homologue produced multiple interesting phenotypes including increased production of GTAs, impaired growth and altered sensitivity to antibiotics. Here we present our current progress on unravelling the underlying regulatory mechanisms using genetic approaches plus qPCR, RNAseq and gene transfer bioassays.

Our results advance our understanding of this fascinating mode of horizontal gene transfer, not only in the model species but also in novel GTA producing species. The *Brucella gafYZ* genes have also been implicated as virulence/fitness factors of unknown function in high-throughput studies, and we also provide insights into their mechanism of action.

B147

Investigating the role of the *S. aureus* stringent response in nasal colonisation.

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Abstract

Staphylococcus aureus is an opportunistic pathogen that commonly colonises the anterior nares of healthy individuals. People may be persistently, or intermittently colonised, with recent carriage studies estimating that 30% of the population are carriers for *S. aureus* at any one time. We hypothesise that the (p)ppGpp-mediated stringent response (SR) pathway may play an important role in nasal colonisation, as this pathway allows bacteria to sense and respond to changes in nutrient availability, potentially facilitating niche adaptation. To test this hypothesis, we have been using a complex liquid culture media, herein termed nasal media, designed to mimic the low nutrient environment of the healthy human sinuses. Using a (p)ppGpp-null strain, which lacks the three (p)ppGpp synthetase enzymes Rel, RelP and RelQ, and is therefore unable to elicit a functional SR, we observed significantly reduced survival in nasal media compared to wild-type. Importantly, this phenotype was not observed in nutrient-rich media. Additional work involving a panel of SR mutants revealed functional redundancy between the (p)ppGpp synthetases, with complementation of the (p)ppGpp-null strain occurring by expression of Rel or RelP, whereas overexpression of (p)ppGpp in wild-type strains did not confer additional growth advantages. Finally, we also determined that SR-enhanced survival in nasal media occurs independently of the master transcriptional repressor CodY, and therefore the mechanism underpinning our findings remains to be fully elucidated. However, preliminary work has highlighted the importance of amino acid availability and RNA-seq is underway to study differences in gene expression between wild-type and (p)ppGpp-null strains.

B148

Belts and braces protection for *M. tuberculosis*; induced auto-phosphorylation blocks toxin $MenT_1$

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Abstract

M. tuberculosis, the causative agent of human tuberculosis, encodes an unusually high number of toxin-antitoxin (TA) systems in comparison to its non-pathogenic relatives, suggesting these systems contribute to the pathogenicity of the organism. One such family of TA systems, $MenAT_{1-4}$, are homologues of the AbiE abortive infection system from *S. agalactiae*, which encodes a putative nucleotidyltransferase toxin, and whose activity is indirectly negated by a cognate antitoxin. Our recent characterisation of the $MenAT_1$ system demonstrated that $MenT_1$ functions as a nucleotidyltransferase that inhibits cellular translation by preventing aminoacyl charging of tRNA acceptor stems. Mutagenesis studies demonstrate that the small N-terminal α-helix of the $MenA_1$ antitoxin, comprising a mere 32 residues, possesses antitoxic properties and efficiently suppresses *in vivo* $MenT_1$ toxicity. Crystallographic studies revealed that $MenA_1$ binds two toxin protomers to form an asymmetric heterotrimeric complex. The crystal structure of $MenT_1$ produced in the presence of $MenA_1$ was then resolved to 2.8 Å, revealing phosphorylation of residue T39, resulting in active site steric occlusion and inversion of electrostatic charge. Biochemical stability assays demonstrated differing nucleotide specificities for phosphorylation and nucleotidyltransferase activities. Using molecular dynamics, we then investigated how $MenAT_1$ complex formation triggers a conformational change to the conserved $MenT_1$ nucleotidyltransferase core, inducing auto-phosphorylation of the toxin. Collectively, our results demonstrate that the $MenAT_1$ TA system represents an atypical type VII TA system whereby neutralisation of cellular toxicity is achieved through a multifaceted inhibition pathway.

B149

The impact of post-transcriptional regulation of α Phenol Soluble Modulins (αPSMs) production on *Staphylococcus aureus* virulence

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a wide range of human diseases due to the coordinated expression of multiple virulence factors. This includes the 4 α Phenol Soluble Modulins (αPSMs), which are amphipathic and α-helical cytolytic toxins that play key roles in facilitating bacteria's survival in host environment. These functions include biofilm formation and structuring, which aids in evading antibiotic treatments and defending against attacks by the host immune system. While the four αPSMs (PSMα1-4) are encoded on an operon, they are not equally expressed. Nanopore sequencing revealed shorter fragments in addition to full-length PSMα operon transcripts, suggesting post-transcriptional regulation of PSMα production. This underscores the need for a detailed understanding of their individual functions and how their production is regulated. We recently found that RsaE, a non-coding RNA, base-pairs with the Shine-Dalgarno (SD) sequences of *psmα3* and *psmα2*. We hypothesise that RsaE contributes to the processing of the αpsm transcript, offering a plausible explanation for their uneven expression levels. To uncover the biological significance of the aforementioned sRNA-mRNA interaction, we are performing phenotypic studies and biofilm structural analyses to quantify the impact of PSMα and RsaE mutants on biofilm formation. Based on our preliminary data, we hypothesise that RsaE specifically regulates PSMα1 and α4 production and alters the stability of the PSMα transcript. We speculate that RsaE-dependent regulation of PSMa toxin production enhances the stability of *S. aureus* biofilms, implying an important role for this non-coding RNA in immune evasion. Results from these studies will be presented.

B150

Bioinformatic analysis of RNA-Seq data relative to Pseudomonas aeruginosa response to antibiotic and oxidative stress

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Abstract

Pseudomonas aeruginosa (PA), a major cause of nosocomial infection, chronic infections and high mortality rates in immunocompromised, cystic fibrosis patients, and burn victims, evades host immunity and often treatment. Analysing genomic data is crucial in comprehending how PA achieves this.

This project investigated changes in PA gene expression upon exposure to antibiotics (meropenem, tetracycline, ciprofloxacin) and oxidative stress (H_2O_2). RNA-seq data (*Gómez-Lozano.et.al.*) was analyzed using statistical tools on bioinformatics platform, Galaxy. Initial steps involved mapping and quantification using 'Bowtie2' and 'featureCounts'. 'DEseq2' assessed differential gene expression between stress and control conditions, followed by 'Goseq' for functional analysis.

Antibiotic stress downregulated *gltBFGKoprB* operon involved in glucose transport and type III secretion system (TTSS) induction whilst upregulating the a*mpC* β-lactamase (PA4110) and *algU/mucA* (PA0762/PA0763) regulators of alginate biosynthesis. Oxidative stress downregulated glycogen metabolic pathways and upregulated bacteriophage-like pyocins spanning PA0614-PA0648, alongside ciprofloxacin. All stressors downregulated a pilus biogenesis protein (PA5041) affecting twitch motility whilst upregulating transcription regulator *lexA* (PA3007) controlling phage mobilization, S-pyocin (PA0985) and a heme-acquisition pathway transcription factor *hxuI* (PA1300).

PA adapts to impaired metabolic pathways causing stress and reducing virulence. Findings indicate reduced PA motility in response to antibiotic and oxidative stress, possibly promoting biofilm formation alongside alginate overproduction, while TTSS downregulation could be an energy redistribution strategy. Pyocins may facilitate DNA transfer among bacteria, aiding survival or resistance. Heme-acquisition could enhance PA's fitness by affecting metabolic and virulence pathways.

Understanding the PA stress response may pave way for novel targeted or combination therapies to effectively combat infection.

B151

Membrane stress response IM30 proteins and their interactions with membranes

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Abstract

Maintaining the integrity of bacterial cell membrane is vital to the sustaining proton motive force (PMF) and cellular homeostasis, essential to bacterial viability. When membrane damage occurs and PMF is undermined, cells respond with membrane "rescue" proteins from the IM30 class, which self-assemble to remodel membranes and stabilise membrane potential. These proteins are universally conserved and structurally analogous to mammalian ESCRT-III proteins. In *E. coli*, this function is performed by the phage-shock proteins (psp), which include effector PspA that dissociates from transcription activator PspF during membrane stress, and translocates to the membrane surface to stabilise membrane integrity. We overexpress and purify *E. coli* PspA and other IM orthologs as recombinant proteins and characterise their oligomeric state in solution, and interactions with membranes using SAXS, CD and solid state NMR, as well as MD simulations. Understanding the mechanism of membrane damage response and repair provides novel insights into the mechanisms of antimicrobial resistance and aids efforts to engineer microbes for biofermentative production of industrial solvents, biopharmaceuticals and biofuels.

B152

Characterising the adaptations of Mycobacterium tuberculosis to fluctuating levels of zinc found during infection

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Abstract

Mycobacterium tuberculosis (*M.tb*) is the leading cause of death by a single infectious agent. *M.tb* must survive in intracellular and extracellular niches *in vivo*, where bacilli must adapt to the changing abundance of metal ions. Transcriptional profiling *M.tb* inside macrophages revealed induction of metal cation pumps responsible for exporting zinc. Botella *et al*. (2011) revealed that *M.tb* is capable of replicating in the high zinc environment of the macrophage that is cidal to other bacteria. Dow *et al.* (2021) showed that *M.tb* can survive in the low zinc environment of the necrotic core of a granuloma, where zinc is sequestered. This suggests that zinc abundance may be a key environmental cue that orchestrates *M.tb* adaptive strategies and zinc may be a modulator of host-pathogen interactions.

This project defines the impact of high and low zinc concentrations on *M.t*b growth and antimicrobial drug efficacy, using *in vitro* growth curves and drug susceptibility assays. This will be combined with transcriptional profiling to characterize adaptations to changing zinc abundance. Ion beam analysis using PIXE will further define the spatial availability of zinc in TB granuloma microenvironments by mapping elemental distribution to a 1µm scale. Understanding the importance of zinc in TB infection offers insights into *M.tb* survival mechanisms and may also unveil potential therapeutic avenues. Targeting zinc-dependent processes in *M.tb* and harnessing the immunomodulatory effects of zinc may pave the way for innovative strategies to treat TB.

B153

The critical role of GadR in the long-term survival of *Listeria monocytogenes* under mild acid stress

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Abstract

The resistance of acid in *L. monocytogenes* heavily relies on the glutamate decarboxylase system, particularly GadD2, which plays a crucial role in the pathogen's prolonged survival in mildly acid-preserved foods. This could significantly impact the public health concerns surrounding *L. monocytogenes* infections. Recently, GadR, identified as a key regulator of GadD2, has been shown to activate GadD2, enhancing the pathogen's ability to survive in highly acidic environments. Building upon this discovery, our research has uncovered that GadR might also play a pivotal role in long-term survival of *L. monocytogenes* in mildly acidic environments. Deleting *gadR* in *L. monocytogenes* resulted in reduced survival rates and the deactivation of GadD2 during prolonged exposure to mild acid conditions. Additionally, our findings suggest that GadR offers protective effects against organic acid stress, hinting at its potential role in *L. monocytogenes'* extended survival in acid-preserved foods.

B154

Rtc-mediated RNA repair activation is regulated *via* the CRISPR-associated Rossmann fold domain of the transcriptional activator RtcR.

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Abstract

The Rtc RNA repair system present in the bacteria *Escherichia coli* and *Salmonella enterica* has been implicated in the development of antibiotic resistance. The system comprises of RNA cyclase RtcA, RNA ligase RtcB and transcriptional activator RtcR, a distant member of the CRISPR-associated Rossmann fold (CARF) domain family. Here we explore the biochemical events underlying Rtc activation *in vitro via* its CARF domain. End labelling of RNA derived from purified RtcR provides evidence for *in vivo* interactions of RNA molecules with RtcR, the extend of which differs between wildtype and N-terminal truncated RtcR lacking its CARF domain. Extracted RNA molecules are ligatable by RtcB *in vitro*, suggesting the presence of 2′,3′-cyclic phosphate (cP) ends which act as substrates for RtcB. *In vitro* transcription and colorimetric ATPase assays reveal a ligand-dependant inhibitory effect being exerted by the RtcR CARF domain upon its adjacent ATPase domain under physiological circumstances. Putative ligands, including oligoadenylates known to activate members of the CARF domain family, and *E. coli* or *S. enterica* RNA extracts, either intact or treated with the endoribonuclease MazF to yield 2'-3' cP ends, were tested against wildtype *E. coli* and *S. enterica* RtcR. Ligands carrying 2'-3' cP ends induced RtcR transcriptional and ATPase activity, indicating an interaction with the CARF domain and subsequent removal of its repressive effect. Current findings suggest that RNA molecules with 2'-3' cP ends activate RtcR *in vitro via* its CARF domain, and further support the potential link between Rtc and tolerance against RNA-targeting antibiotics.

BLOCK B

Session : Biofilm Prevention and Control

B159

The antimicrobial effects of chlorhexidine on caries-causing bacteria (planktonic cells vs biofilms).

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Abstract

Streptococcus mutans and *Lactobacillus casei* are causative agents of dental caries and tooth plaque in humans. To treat oral diseases, antimicrobial mouth rinses containing chlorhexidine (CHX) are routinely used. Unfortunately, these oral antiseptics are contributing to antimicrobial resistance. This study aimed to determine the increase in minimum inhibitory concentrations (MICs) of *S. mutans* and *L. casei* following repeated exposure to CHX digluconate (0.02%) *in vitro.* MIC values increased ≈ 2-fold by passage 15 for both species. Thereafter, cross-adaptation to common oral antiseptics (PerioGard, Corsodyl Original and Corsodyl Daily) was examined. CHX-adapted bacteria demonstrated a higher tolerance to CHX-containing mouthwashes compared to non-adapted strains. Additionally, a qualitative assay exposed a dual-species biofilm model (*S. mutans* and *L. casei*) to different concentrations of CHX digluconate (1.56 ug/ml – 200 ug/ml) and Corsodyl (15.6 ug/ml – 2000 ug/ml). Cell survival was determined using Dey-Engley neutralising broth and biofilm-forming capacity post-treatment was analysed. Bacterial survival occurred across all CHX and Corsodyl concentrations, and there were no statistically significant differences in biofilm forming capacities between adapted and non-adapted strains. This study showed that caries-causing bacteria can develop resistance in various concentrations of CHX. Moreover, biofilms demonstrated a greater tolerance to oral antimicrobials compared to planktonic cells.

B160

Characterising antibiotic tolerance and biofilm growth of *Pseudomonas aeruginosa* in cystic fibrosis

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Abstract

Pseudomonas aeruginosa forms complex biofilms in the airways of people with the genetic condition cystic fibrosis (CF). These are almost impossible to eradicate, and the results of diagnostic antibiotic susceptibility tests are a very poor predictor of which agents may have efficacy *in vivo*. The biofilm matrix constructed in the CF airways by *P. aeruginosa* appears highly refractory to antibiotic entry, and may be more protective than the matrix that this bacterium produces when grown in standard laboratory conditions. Additionally, the unique environment within CF airways cues specific changes in the *P. aeruginosa* transcriptome which could contribute to high-level antibiotic tolerance. In this talk, I will give an overview of work by the Harrison lab and our collaborators that starts to dissect the contributions of changes in gene expression, metabolism and matrix construction to the antibiotic tolerance of *P. aeruginosa* in CF.

Working with synthetic CF sputum medium and an *ex vivo* model of biofilm in CF bronchioles, we have used biofilm eradication assays, matrix degradation assays, transcriptomics, metabolomics and microscopy to explore this question. We demonstrate that the biofilm matrix produced by *P. aeruginosa* in our CF model is indeed very difficult for antibiotics to penetrate, and structurally different from that produced in standard lab models; however, biofilm-deficient mutants can show comparable antibiotic tolerance to wild-type bacteria when grown in the model. This appears to be due to changes in the expression of some well-understood antibiotic resistance determinants, as well as more subtle changes in metabolism.

B161

A promising support for last-resort antibiotics – A new 2-aminooxazole derivative enhances the anti-biofilm effect of colistin against multidrug-resistant *Acinetobacter baumannii*

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Abstract

ESKAPE pathogens are causative agents of healthcare-associated infections with reduced treatment options and a broad spectrum of virulence and resistance factors e.g., biofilm formation. *Acinetobacter baumannii* is categorized among pathogens with the highest priority for which research of novel antibiotics is desperately required.

Based on our preliminary data, we hypothesize that a recently synthesized chlorinated 2-aminooxazole derivative, AB15, would stand as an auspicious agent in the increasingly challenging fight against resistant bacterial pathogens. Therefore, in this study, we aimed to comprehensively describe an antibacterial action of our candidate compound.

The AB15 revealed a promising bactericidal effect against clinical isolates of the ESKAPE group, especially *A. baumannii* (15. 63-62.5 µM) using standard microdilution broth method. Non to low toxicity was assessed *in vitro* using HK-2 cell line. The non-toxicity of AB15 was also confirmed *in vivo* by monitoring the survival of *Galleria mellonella* model organism after an intra-haemocoel and per-oral administration. Observed in checkerboard assays, AB15 reveals an additive effect to some clinically relevant antibiotics. Moreover, no antagonistic effect of AB15 was registered in combination ratios with any of the studied drugs. In mutual interaction with a last-resort antibiotic, colistin, AB15 shows synergistic action against a multidrug-resistant *A. baumannii* clinical isolate. In addition, AB15, in combination with colistin, contributes to significantly higher anti-biofilm activity against a biofilm consortium formed by the biofilm producing *A. baumannii* isolate.

In conclusion, AB15 can be designated a valid antibiotic adjuvant as it meets all key attributes of such compound.

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B162

Prolonged exposure of *Lm* biofilms to BC selects for disinfectant resistance and other traits related to biofilm fitness.

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Abstract

Listeria monocytogenes (Lm) is the third-leading cause of death attributed to foodborne pathogens and is associated with contamination of food at production facilities. Biofilm formation is one of the mechanisms that *Lm* possess to survive food industry disinfectants and persist in manufacturing environments. Prolonged exposure to sub-inhibitory concentrations of the commonly applied disinfectant benzalkonium chloride (BC) results in emergence of BC-tolerant *Lm* strains. However, there is limited understanding of how *Lm* evolve in the context of biofilms in response to sub-inhibitory concentrations of BC. Here we used a biofilm evolution model, which pairs prolonged exposure to sub-inhibitory BC concentrations with whole-genome sequencing (WGS) to identify mutations in genes and pathways that improve the overall fitness of *Lm* in the context of biofilm formation and tolerance towards BC. The biofilm evolution experiment spanned 30 passages, followed by a range of phenotypic assays to assess the change in *Lm* fitness and BC tolerance over the evolution period, including determination of MICs, cell attachment to stainless steel, growth dynamics and crystal violet assays. WGS was performed on a group of strains from early, middle, and late stages of the biofilm evolution experiment. Genomes were analysed to detect key genetic traits correlating to evolution model. Regions of interest associated with increased tolerance to BC were identified. Currently, we are in the process of connecting phenotypic and genotypic data to identify more genes/pathways responsible for improved fitness and increased tolerance to BC.

B163

Impact of fungal pathogens and environmental conditions on the antimicrobial tolerance of Pseudomonas aeruginosa biofilms in the cystic fibrosis context

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Abstract

A range of in vitro biofilm models are currently used to evaluate the effectiveness of antibiotics used to treat infections in PwCF (people with cystic fibrosis). Most do not take into account the natural of the CF environment or the inter kingdom polymicrobial interactions within the CF lung, sometimes leading to high rates of antibiotic failure. We have developed a highly versatile tri-species colony polymicrobial biofilm model to assess the effects of CF environmental conditions and the presence of fungi on the response of P. aeruginosa to a range of commonly used antibiotics. The results showed that the MBIC50 and MBIC90 of meropenem, colistin, tobramycin, and ciprofloxacin against P. aeruginosa were significantly decreased when A. fumigatus was the fungal representative compared to C. albicans in the polymicrobial setting. Changing environmental conditions also showed significant variations in both biofilm structure and antimicrobial tolerance. Furthermore, treatment of P. aeruginosa polymicrobial biofilms resulted in a significant CFU increase of co-cultured microbes highlighting the impact of antibiotics on altering population dynamics within polymicrobial biofilms.

These results highlight the need to considerer the polymicrobial nature of biofilms and the changes in the CF environment when establishing the effectiveness of antimicrobials against P. aeruginosa.

B164

Mining the nanotube-forming Bacillus amyloliquefaciens MR14M3 genome for determining anti-Candida auris and anti-Candida albicans potential by pathogenicity and comparative genomics analysis

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Abstract

Candida auris is an emerging multidrug-resistant fungal pathogen. One of its concerning aspects is its ability to survive on various surfaces, particularly in the healthcare environment, leading to its persistence and transmission. The identification of an effective treatment for this emerging condition is a critical solution that necessitates immediate attention. A bacterial strain with anti-Candida auris activity was isolated and identified using 16S rRNA gene sequencing. The native isolate IRMC143 has the ability to inhibit C. auris (zone of inhibition: 25 mm). The whole genome was sequenced to identify biosynthesis-related gene clusters. The comprehensive genome analysis using bioinformatics tools revealed that the assembled genome of Bacillus amyloliquefaciens (strain number IRMC143) has 5,722 protein-coding sequences (CDS). Additionally, analysis with antiSMASH software resulted in the identification of 13 cluster regions, each with a unique variety of genes. Five clusters encode the biosynthesis of non-ribosomal peptide (NRP), two clusters encode the synthesis of polyketide synthase (PKS), and one cluster encodes the synthesis of saccharide. Two clusters code for the biosynthesis of hybrid NRP/PKS compounds. Utilizing the NCBI blast tool for gene mutation identification within each cluster, it was shown that the surfactin region coding for the srfaa gene exhibited both insertion and point mutation. These mutations could potentially confer antifungal activity.

B165

Understanding the Role of Biofilms in the Failure of Voice Prosthesis Implants

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Abstract

Silicone tracheoesophageal valves are routinely fitted in patients following surgical removal of advanced head and neck cancers; however, they are routinely colonized by biofilms in vivo which leads to high failure rates. The failure rate of voice prosthetics (VPs) leads to the patient's dependency on regular clinical intervention to replace failed VPs, and almost daily use of high-dose antimicrobials to control the colonization of the implant. Despite these burdens, the composition and impact of colonizing pathogens on VP integrity are poorly understood. We present a multi-faceted workflow to determine the bioburden on explanted VPs to inform better clinical practice and manufacture of tracheoesophageal implants. We used a combination of reflection and fluorescence confocal microscopy to map the surface of the VP and reveal the presence of bacterial and fungal biofilms. The bioburden was determined by quantifying the levels of different biofilms on the surface, and deterioration of the VP surface was monitored by reflection imaging. We show that VPs were colonized by both fungal and bacterial communities, which form discreet biofilms over the surface. The spatial organization is also linked to the degradation of the VP surface, where we show that fungal biofilms invade and perforate the surface of VPs. The new insights show that complex polymicrobial biofilms are responsible for implant failure. Furthermore, our findings indicate that more tailored antimicrobial therapies and higher standards of implant manufacturing are required to lower the bioburden of VPs.

B167

The influence of the chronic wound microenvironment on antibiotic-bacteriophage co-therapy on E. coli biofilms

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Abstract

Biofilms in chronic wounds, e.g. Diabetic foot ulcer, are a significant healthcare burden due to their increasing prevalence, emerging antibiotic resistance, and complex interactions with distinct microenvironments. In this study, a medium which accurately mimics the chronic wound microenvironment of Diabetic foot ulcer was used to study the effect of antibiotic (streptomycin) and bacteriophage co-therapy on E. coli biofilms. Biofilms will be analysed quantitively (biofilm biomass) and qualitatively (light microscopy) to determine changes in biofilm growth. Results will be contrasted against those obtained using a rich growth media (TSB), to demonstrate the importance of considering, and when possible, mimicking, the microenvironment when working with biofilms in vitro. The aims of this project are to advance research in biofilm co-therapy in chronic wounds by investigating the impact of microenvironment specific media, and to develop recommendations for further research into how chronic wound media influences biofilm formation.

B168

3 Dimensional Tracking of Pseudomonas aeruginosa to Probe Effects of Membrane Active Antimicrobials

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Abstract

Introduction: Swimming motility in *Pseudomonas aeruginosa* occurs via a single polar flagellum. This is powered via a motor embedded within the cell envelope that couples an electrochemical gradient with mechanical rotation. The link between swimming and membrane potential perturbations could be exploited to visualise membrane active antimicrobial activity through tracking bacterial motility. Here digital holographic microscopy (DHM) was used to track of a population of bacterial cells individually in 3D. Antimicrobials were adsorbed to surfaces, exposed to bacterial suspensions and then monitored for changes in motility over time.

Methods: Chlorhexidine, cetylpyridinium chloride (CPC), o-cymen-5-ol and formulated chlorhexidine (FCHX) elution from surfaces was quantified via reduction in constitutive bioluminescence of *P. aeruginosa* CTX::*tac'-luxCDABE* as an indicator of membrane damage. Exponential and stationary phase wild type (WT) and RpoS negative (RpoS-) cell motility was visualised using DHM with 1000 frame (18 s) image streams being taken every 10 min over 1 h. Bacteria were tracked using bespoke LABVIEW scripts and reconstructed using MATLAB.

Results: Bioluminescence decreased over time with chlorhexidine and FCHX for exponential WT and stationary RpoS- cells. This corresponded with changes in bacterial trajectories exposed to chlorhexidine and FCHX which showed increased numbers of sharp turns, quantified as an increase in tortuosity. Stationary WT cells only responded to FCHX surfaces. CPC and o-cymen-5-ol showed no change in luminescence (no elution) and no change in tortuosity at the concentrations tested.

Conclusions: Chlorhexidine and FCHX readily desorb from surfaces, dissipating membrane potential which in turn impacts on bacterial motility.

B169

Emergence of new pathogens in hospitals – the cost of ever-increasing hygiene?

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Abstract

The majority of Healthcare Acquired Infections (HAIs) are caused by human commensal bacteria. Efforts to reduce infection rate have met with incremental success. Zero infection rate is often the target, though unlikely, and increasing hygiene is subject to the law of diminishing returns. There is now attention toward ubiquitous bacteria found in water and soil that are innately resistant to many common antimicrobials and antibiotics, are highly metabolically flexible, can be found in water used for drinking and washing, and can be opportunistic pathogens particularly in the immunocompromised. Examples such as *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* are best known. This study aimed to determine whether hygiene may paradoxically drive the spread of *P. aeruginosa* in clinical environments. Supply tap water was found to support very little growth or biofilm production in inoculated *Pseudomonas* spp. due to lack of an energy source and bioavailable carbon, yet the bacteria were highly persistent. When hospital hand wash was added at very dilute concentrations in a drain model, previously undetectable cetrimide-resistant bacteria (and inoculated *P. aeruginosa*) underwent a "bloom" and formation of thick surface biofilm that is likely to further resist disinfection. These bacteria may have a "persist-bloom-persist" life cycle driven by catabolism of components in soaps and disinfectants, causing them to spread, persist, and produce significant biofilms even in clean environments. Such a cycle might be broken by greater emphasis on disinfecting clinical drains and sink traps, and disinfection with antimicrobials that leave no residue such as hydrogen peroxide.

B170

Investigating the antibiofilm potential of Bald's eyesalve-derived natural product cocktail against chronic wound bacterial pathogens

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Abstract

Biofilm infections are of great concern because they are often resistant to antibiotics and require up to 1000X the concentration of antibiotics needed to clear planktonic bacteria. Chronic wound infections are good examples of biofilm infections and cost the NHS around £5Bn yearly. We aim to develop a natural product cocktail derived from a medieval infection remedy – "Bald's Eyesalve" – with antibiofilm efficacy in chronic wound infections. The original remedy is a combination of four chemically complex natural ingredients. We found that the biofilm eradication activity of Bald's eyesalve can be recapitulated by a semi-synthetic cocktail comprising a compound purified from one ingredient (compound A) and a second ingredient from the remedy (ingredient B). We report that the semi-synthetic cocktail has good activity against both planktonic and biofilm-associated populations of *Staphylococcus aureus, Acinetobacter baumannii,* and *Pseudomonas aeruginosa* in standard lab media and host-mimicking models. The cocktail showed >4-log killing against *S. aureus* (methicillin-susceptible strain) and *A. baumannii* in an *in vitro* soft tissue biofilm model. We also confirmed that the cocktail has better activity than its individual components, especially in the soft tissue biofilm model. We also examined the interaction between cocktail components and other antimicrobials or potentiators. Combining the cocktail with potentiators or other antimicrobials improved activity against *P. aeruginosa* and methicillin-resistant *S. aureus* in *in vitro* soft tissue biofilm model. We have shown that this natural product cocktail could potentially be developed into a good treatment for chronic bacterial biofilm infections, especially chronic wound infections.

B171

Modelling CAUTI for the application of developing novel antimicrobial catheter coatings with inverse-vulcanized sulfur polymers

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Abstract

Indwelling urinary catheters are amongst the most commonly deployed medical devices, and are implicated in up to 80 % of urinary tract infections (UTIs). Catheter-associated urinary tract infections (CAUTIs) are often accompanied by more complex upper UTI symptoms and an increased risk of secondary bloodstream infections. Biofilm formation is an integral stage in CAUTI pathogenesis, with the urinary catheter providing the initial site of bacterial adhesion, as well as protection from host immune factors.

Our previous work highlighted the promising application of inverse-vulcanized sulfur polymers as antimicrobial surface coatings, with preliminary data showing a reduction in viable cell counts and biofilm formation of key uropathogens *P. aeruginosa* and *S. aureus* following incubation with sulfur polymers. In this work, we describe the potential application of inverse-vulcanized sulfur polymers as novel antimicrobial urinary catheter coatings. We detail methods for the functionalisation and coating of polydimethylsiloxane (PDMS; silicone) with bulk polymer and water dispersible sulfur polymer nanoparticles, as well as the rationale behind the selection of comonomers for inverse-vulcanization of sulfur.

Additionally, we utilize models which simulate biofilm formation in the catheterized urinary tract to study the antimicrobial and antifouling properties of sulfur polymers in CAUTI. We describe modifications to the 3D-printed Flexipeg biofilm device for the high-throughput screening of prospective sulfur polymer coatings, prior to trialling coated catheters in *in vitro* bladder models with a panel of clinical UTI-associated *E. coli* isolates. These data collectively highlight the potential of inverse-vulcanized sulfur polymer catheter coatings as a promising tool to help combat CAUTI.

B173

Exploring antibiofilm and synergistic interactions of cationic polymers.

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Abstract

As a result of antimicrobial resistance, we edge closer to a post-antibiotic era and the development of novel antimicrobials is urgently required. Biofilm formation plays a significant role in bacterial persistence and resistance to antibiotics, with up to 80% of bacterial infections being linked to biofilm forming bacteria. An approach to treating biofilms is the use of antimicrobial peptides, small peptides that target bacteria. However, their widespread clinical application is limited, partly due to cost of production. Polymer chemistry, specifically reversible-addition fragmentation chain transfer (RAFT) polymerisation, can be utilised to synthesise an alternative polymer-based system, mimicking key aspects of the natural peptides, in a very scalable process. These polymers are proving to be a promising platform for the development of novel antimicrobials as their antimicrobial effects and toxicity can be tuned, while keeping production costs low. We synthesised ammonium containing polymers, previously shown to exhibit antimicrobial and anti-biofilm activity against *Pseudomonas aeruginosa*. To better understand the extent of the polymer activity we tested the antimicrobial effects using MIC assays against a panel of 5 bacterial and fungal pathogens of concern, in different media to mimic clinically relevant conditions (synthetic wound fluid, airway synthetic liquid and synthetic cystic fibrosis media). We then determined the antibiofilm properties of the polymers by using a Calgary device. Following this, promising polymers were taken forward to investigate their potential synergistic activity with existing antimicrobials. Future work will investigate synergistic combinations of polymer and antimicrobial to test for anti-biofilm activity in high validity biofilm models.

B174

Development of a liquid polymicrobial biofilm model for Cystic Fibrosis therapeutics

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Abstract

In the lungs of a person with cystic fibrosis (CF), microbes colonize and form biofilms containing multiple species of bacteria and fungi. Interactions between organisms are overlooked when determining the effectiveness of antimicrobials. The lung environment is also altered in CF and CF sputum contains high levels of mucin, extracellular DNA and amino acids. The chemical environment can affect the resistance of bacteria in this niche.

A polymicrobial biofilm model was developed using a CF-isolate of *Pseudomonas aeruginosa* , *Staphylococcus aureus* and *Candida albicans* in synthetic sputum, across various oxygen concentrations to mimic conditions in the centre of biofilms, and within microaerophilic pockets in the lungs. DNase was also trialled in the model to observe the effects on the resistance of the biofilms against meropenem and colistin.

Antibiotics were applied following 24 h biofilm growth and studied at 48 h. Using reduction in bacterial load and metabolic activity as readouts, very high levels of resistance to meropenem and colistin were observed for *Pseudomonas* across all oxygen concentrations, demonstrating the adaptability of the biofilms to survive in hostile environments.

Ongoing work involves studying the impact of complex polymicrobial biofilms on resistance to these antibiotics and novel therapeutics, including phage. Transcriptomics will be utilised in order to refine models to more closely capture the gene expression pattern of *P. aeruginosa* that has been observed during chronic respiratory infection.

This work contributes to a wider project (PIPE-CF), that aims to develop a robust preclinical framework for the development of novel CF antimicrobials.

B175

Investigating the antimicrobial potential of Irish monofloral honeys for wound healing applications

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Abstract

The spread of multi-drug resistant bacteria is necessitating research into alternatives to antibiotics to treat infections and improve wound healing outcomes. Due to the success of manuka as a therapeutic honey, varieties of monofloral honeys are being investigated globally for their therapeutic activity. Significant total phenolic content (TPC), indicative of potential antimicrobial action, has been reported for Irish heather and ivy honey (Kavanagh, *et al*. 2019) making these honeys ideal candidates for investigation.

The antimicrobial potential of twelve Irish monofloral (heather and ivy) honeys, against *S. epidermis* DSM20044*, S. aureus ATCC25923, E. coli ATCC25922, P. aeruginosa* NCTC10662 was investigated (MICs and antibiofilm activity). Results were evaluated against manuka and a synthetic honey, based on EUCAST guidelines. Effects on *S. aureus* and *P. aeruginosa* biofilm formation and eradication were assessed.

Both heather and ivy honey (25% v/v) exhibited greater inhibitory activity than synthetic honey, against all four bacteria. Heather honey showed comparable MIC values to manuka honey (6.25-12.5% (v/v)). Heather and manuka honey inhibited *S. aureus* biofilm formation at 3.125% and 6.25% (v/v) respectively, and *P. aeruginosa* biofilm formation at 25% (v/v). Ivy honey inhibited the formation of *S. aureus* biofilms and *P. aeruginosa* biofilms *at* 25% (v/v).

This research indicates that both Irish ivy and heather honey may exhibit antimicrobial activity against key wound pathogens, with some effects comparable to manuka honey. This warrants further exploration of these honeys for therapeutic application in wound healing formulations.

B176

Exploring the Efficacy of Lipids as Antimicrobial and Antibiofilm Agents against *Streptococcus mutans*

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Abstract

Biofilms are responsible for oral diseases such as dental caries and are more resistant to treatments.

Saturated and unsaturated medium and long chain fatty acids (FAs) were screened for antimicrobial activity against *Streptococcus mutans* ATCC 25175. Effects on *S. mutans* growth was determined at 250, 100, 50 and 10 µg/ml. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined and impact of FAs on bacterial metabolic activity was determined using the Resazurin assay. Synergistic activity of FAs against *S. mutans* was also evaluated. Minimum Biofilm Inhibition Concentration (MBIC) was determined using crystal violet staining. LDH and XTT assays were employed to investigate oral cavity cell toxicity and proliferation.

The MIC for saturated Undecanoic (C11), Lauric (C12), and Myristic (C14) acid was determined at 50 µg/ml. At 10 µg/ml Oleic (C18:1), Linoleic (C18:2), y-Linoleic (C18:3), Eicosapentaenoic acid (EPA, C20:5) reduced bacterial growth. Docosahexaenoic acid (DHA, C22:6) prevented bacteria growth at 10 µg/ml, reduced metabolic activity, inhibited biofilm formation, and displayed a log reduction value of 6. Combining most effective FAs at 10 µg/ml displayed bacteriostatic effects and improved log reduction compared to when used alone. At 10 µg/ml y-Linoleic, EPA and DHA displayed less cytotoxic effects than chlorohexidine, α-Linoleic displayed most protective effects on TR146 cells.

Unsaturated long chain FAs with one or more double bonds produced significant antimicrobial activity against *S. mutans*. Most promising FA is Omega 3; DHA, while combining AMLs demonstrates synergistic activity and may reduce cytotoxic effects. AMLs have potential to be used as novel antibiofilm agents against *S. mutans*.

B177

Are bacteria building toxic biofilms in our gut?

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Abstract

Sulphate-reducing bacteria (SRB) are a group of anaerobic microorganisms that commonly exist within biofilms. During anaerobic respiration, SRB produce the corrosive and genotoxic hydrogen sulphide gas as a by-product of dissimilatory sulphate reduction. In the environment, SRB contribute to corrosion of metal structures, causing heavy financial losses for oil and gas industries. In humans, SRB blooms have been linked to Parkinson's disease and inflammatory gut diseases such as ulcerative colitis and inflammatory bowel disease. However, little is known about the role of SRB biofilm formation on gut colonisation in humans. To address this knowledge gap, 23 SRB strains were isolated from the gut microbiota of healthy people over 60 years and their genomes sequenced. Of these strains, 5 were identified as *Desulfovibrio desulfuricans*. Based on phylogenomic analysis, the *D. desulfuricans* strains clustered into two distinct clades, indicating that reclassification of the species is necessary. Biofilm-forming capacity, measured using crystal violet staining on a polystyrene surface, identified 2 biofilm-forming and 7 non-biofilm forming strains of *D. desulfuricans*. The capacity of these bacteria to form biofilm was not linked to their phylogenomic classification. Future comparative genomics and transcriptomic analyses will help identify the genes that triggered biofilm formation in these gut SRB isolates. Understanding the molecular mechanism underpinning biofilm formation and dispersal of gut SRB can inform strategies to control their population in the gut to promote human health.

B178

The impact of quorum-sensing mediated autolysis on *Pseudomonas aeruginosa* biofilm formation and antibiotic tolerance

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Abstract

The opportunistic bacterial pathogen *Pseudomonas aeruginosa* is a leading cause of antimicrobial resistance-related deaths, and novel antimicrobial therapies are urgently required. *P. aeruginosa* infections are difficult to treat due to the bacterium's propensity to form biofilms, where cells aggregate to form a cooperative protective structure. Autolysis, the self-killing of bacterial cells, and the bacterial cell-to-cell communication system, quorum-sensing (QS), play essential roles in biofilm formation. Strains of *P. aeruginosa* that have lost the LasI/R QS system commonly develop in patients, and previous studies have characterised distinctive autolysis phenotypes in these strains. Yet, the underlying causes and implications of these autolysis phenotypes remain unknown. This study confirmed these autolysis phenotypes in the PA14 QS mutant strains, ΔLasI and ΔLasR, and investigated the consequences of QS loss and associated autolysis on biofilm formation and antibiotic susceptibility. QS mutants exhibited delayed biofilm formation but ultimately surpassed the wild-type (WT) in biofilm mass. However, these biofilms did not contain higher live cell numbers than the WT and were more susceptible to certain antibiotics, indicating the lack of QS functioning altered biofilm structure and antibiotic tolerance. Artificial supplementation of ΔLasI with QS signal molecule (autoinducer) restored the strain's QS system without the associated costs of QS, enabling ΔLasI biofilm live-cell numbers to surpass the WT in treated and untreated conditions. The altered patterns of autolysis, biofilm formation and antibiotic tolerance associated with QS-loss advocates the use of QS-inhibitors in anti-biofilm therapies, with further research required to fully elucidate how and why these autolysis phenotypes occur.

B179

Investigating the role of the *Staphylococcus aureus* Type VII Secretion System in biofilm formation, degradation, and bacterial competition

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Abstract

Staphylococcus aureus often switches to growing as a biofilm during chronic infection. This results in significant clinical implications; such infections are difficult to treat, especially as tolerance to antibiotics arises from this growth phenotype. The Type VII secretion system (T7SS) is one way by which the pathogen *S. aureus* transports proteins (substrates), including toxins to target competitor bacteria, out of the cell (1,2). It has been shown in *Bacillus subtilis* that T7SS substrates only offer a competitive advantage during biofilm, not planktonic, growth (3). Preliminary data shows that the T7SS is not required to establish a biofilm in *S. aureus* RN6390, but its presence does confer a competitive advantage over mutants lacking the T7SS locus in co-culture biofilms. Additionally we have shown that a T7SS substrate, the nuclease toxin EsaD, is able to degrade established *S. aureus* biofilm. Moving forward, we aim to establish if specific components and substrates of the T7SS are involved in these processes to outcompete competitors in a biofilm context, and to explore how these processes can be exploited to prevent formation or degrade biofilm.

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B180

Drug Resistance Profile and Whole Genome Analysis of Hydrogen Sulphide (H_2S) Producing Bacteria in Poultry Settings: A Step Towards Management

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Abstract

Hydrogen sulphide-producing microorganisms are common in the poultry industry. Exposure to the noxious H_2S gas can irritate the respiratory system and eyes, even at low concentrations. In this study, environmental swabs, faecal, carcass, and feather samples were obtained from poultry processing facilities in the United Kingdom. From each sample, Xylose Lysine Deoxycholate and Hektoen Enteric agars were used for selective isolation of H_2S-producing bacteria. The antibiotic susceptibility profile of each isolate to ampicillin, ceftriaxone, ceftazidime, ertapenem, imipenem, meropenem, gentamicin, streptomycin, azithromycin, tetracycline, ciprofloxacin, nalidixic acid, pefloxacin, co-trimoxazole, and chloramphenicol was determined using Clinical and Laboratory Standards Institute guidelines for disc concentration and result interpretation. The whole genome of all isolates was sequenced for molecular characterization and identification of antimicrobial resistance genes (ARGs). A total of 28 H_2S-producing bacteria were recovered and phylogenetic analysis identified 14% of the isolates as *Salmonella enterica,* while 32% and 54% belonged to *Citrobacter (braakii and werkmanii) and Proteus mirabilis*, respectively. All isolates were resistant to at least two antibiotics except for one strain of *S. enterica*. Interestingly, one strain of *C. braakii, P. mirabilis,* and *C. werkmanii* showed resistance to 7 (AMP-CN-S-AZM-TE-CIP-PEF), 3 (S-TE-CIP), and 2 (AMP-CIP) antibiotics belonging to 5, 3, and 2 antibiotic classes, respectively. The genomes of these isolates contained 10, 6, and 33 ARGs, respectively. While this project is ongoing, our current data provides insight into potential intervention strategies and emphasises the need for monitoring H_2S-producing microorganisms in poultry settings to ensure food quality and staff safety.

B181

The comparison of different nutrient conditions in the formation of methicillin-resistant S*taphylococcus aureus* and *Candida albicans* dual-species biofilms *in vitro*

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Abstract

Microorganisms, including multidrug-resistant ones, prefer a lifestyle in complex communities called biofilms, which are mostly polymicrobial. Especially due to the *quorum-sensing* signals and the presence of the biofilm matrix, participants are more protected against hostile conditions, and hitting them effectively is much more complicated. To streamline, the evaluation of new required anti-biofilm therapeutic strategies, adequate biofilm models are needed.

The goal of this study was the establishment of *in vitro* nutrition conditions closely mimicking the host environment to produce appropriate dual-species biofilms from medically important bacterium methicillin-resistant *Staphylococcus aureus* and yeast *Candida albicans* with similar attributes to the microbial communities formed *in vivo*.

Biofilms were formed in four various cultivation media: Tryptic soy broth, RPMI 1640 with and without glucose, and Lubbock medium. Different amounts of host effector molecules such as human plasma or sheep red blood cells supplemented all media. To compare formed consortia, key biofilm attributes, total biofilm biomass, or the biofilm's ability to withstand exposure to selected antimicrobial drugs was evaluated. Next, the principal matrix biomolecules, or representation of individual microorganisms were quantified.

In summary, although the Lubbock medium didn't mimic the host environment the most, it provided the most appropriate amount of nutrients regarding the biomass structure and the highest degree of tolerance to selected antimicrobials with the evident contribution of the biofilm matrix. Additionally, one of the potential targets of a complex antibiofilm strategy, carbohydrates, was revealed as the prevailing molecules in the matrices regardless of the cultivation media.

This study was supported by the Ministry of Health of the Czech Republic, grant nr. NU21-05-00482.

B182

Assessing the effects of silvers on antibiotic resistant and biofilm producing wound associated bacteria.

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Abstract

As antibiotic options become limited due to resistance and a lack of new antibiotics entering the market, there is a move towards metals to aid in treatment. Metals, such as silver (Ag) have been long known for their activity against bacteria, viruses, and some fungi. Silver exhibits antimicrobial activity in different forms, from nanoparticles, to being attached to antibiotics, such as silver sulfadiazine, which is approved for direct use on the human body as an ointment for wound treatment. Other forms of silver (e.g. nanocrystalline) are incorporated into dressings which release elemental silver into wounds. We aimed to identify promising silver compounds for incorporation into an alginate-based hydrogel, which will later be assessed for effectiveness as an experimental wound dressing.

Compounds of interest were screened for their minimum inhibitory concentration (MIC), against 12 strains of bacteria and one fungus, in cation-adjusted Mueller-Hinton broth (caMHB) and synthetic wound fluid (SWF). Biofilm inhibitory concentrations (BIC) were then determined via crystal violet staining and an adapted protocol from Moskowitz, et al., (2004).

Silver sulfadiazine had an MIC of 32-256µg/mL in caMHB and 4-128µg/mL in SWF, as expected. MIC results for silver nanoparticles were inconclusive, as manufacturer concentration was a limiting factor. Colloidal silver did not affect bacteria, this could be due to the size distribution (determined through TEM). Biofilm growth curves identified promising strains for BIC. Overall, we have found that different forms of silver have promising MICs and that host-mimicking media can increase or decrease silver efficacy compared with caMHB.

B183

Biocide Use in Product Type 6 In-can Products: Identifying Contaminating Spoilage from Microorganisms in Different Matrices

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Abstract

The use of biocides in the treatment and prevention of biofilm-forming microorganisms (BFM) is a current area of focus, with the financial cost of biofilms estimated at almost $4,000 billion a year. For example, biocides can be added to products stored in contained environments (e.g.paint), known as in-can preservation. As such, methods that can be used to determine efficacy of biocides in such products are required. A scoping review under PRISMA guidelines was completed to quantify the microbial challenge relating to biofilm spoilage, the storage conditions (such as pH or temperature) and understand approaches to efficacy in the assessment of biocides in different matrices that are applicable to biocide use for in-can preservation. Overall, literature exploring this problem was limited, but did highlight some microorganisms responsible for in-can contamination with *P. aeruginosa* being the main organism of interest, followed by *Legionella* species and *E. coli* due to presence in production and storage of in-can products. Matrices under investigation included paint, textiles, adhesives, wood and paper production, with work to develop a method to assess different in-can matrices with known microbial contaminants undertaken. The ability for biofilm to establish in these matrices was quantified using plate counts and microscopy, with diversity of biofilm formation on an environment-by-environment basis. The results contribute to the understanding of biofilm formation in in-can preservation and can lead to improved strategies for biocidal action in different matrices. The study identifies key microorganisms of focus and inform a protocol in the assessment of biocides in different matrices.

B184

How does the addition of fungi to bacterial biofilm impact the efficacy of biocides and antimicrobial surfaces?

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Abstract

Extensive research has explored bacterial biofilms and their formation. However, in natural environments, biofilms frequently comprise a combination of fungal and bacterial species, leading to increased co-aggregation after initial surface colonisation. Understanding these interactions is crucial for mitigating biofilm growth, which has significant implications in various industries beyond the clinical setting, including fracking, paint manufacturing, water distribution, water cooling systems and food processing. Therefore, there is a pressing need to develop standardised testing methodologies for antibiofilm materials such as biocides and antimicrobial surfaces, that consider fungal-bacterial interactions within the biofilm matrix. The present study aims to grow mixed fungal-bacterial biofilms using a standardised methodology and test survival after treating with different biocides for different amounts of time. To validate our methodology, confocal laser scanning microscopy (CLSM) was used to image fungal-bacterial biofilm grown on different surfaces and in well plates. Biofilm thickness as a measure of biofilm growth was assessed using image analysis software. The results indicated that biofilm thickness was influenced by the presence of different fungal and bacterial microorganisms within the biofilm. These findings highlight the importance of considering the interactions between fungi and bacteria in biofilm formation and suggest that standardised testing methodologies should be representative of end-user environments to accurately assess the efficacy of antibiofilm materials.

B185

A Mathematical Approach to Model Signal Propagation in Biofilms

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Abstract

Biofilms are complex microbial communities encapsulated within an extracellular polymeric substance. Crucial to their survival and functioning is the transport of signalling molecules within this matrix. Within the biofilm, specialised water channels facilitate the express transport of nutrients and signalling molecules. To modulate biofilm communication effectively, it is essential to understand the underlying signalling mechanisms. A deeper insight into the biofilm's communicative interactions would facilitate such modulation, allowing for the improvement or interruption of these processes.

The communication system in biofilms is inherently noisy due to the stochastic nature of molecule propagation. This randomness is a critical factor in determining the trajectory of autoinducers and which bacteria they reach. Considering the biofilm's porosity and the non-uniform diffusion facilitated by water channels, we have developed a mathematical model that describes the propagation of autoinducers within mature biofilms. This model is based on Green's function for concentration and is validated through simulation. By incorporating the probability of signalling errors over varying distances between bacteria, our model accounts for the diffusion dynamics within the biofilm matrix. Furthermore, by applying the Blahut-Arimoto algorithm to our formulation, a theoretical framework for the maximum reliable information transmission rate between bacteria situated at different points within the biofilm can be established.

Our findings have significant implications for the development of strategies aimed at either inhibiting or enhancing biofilm functionality. This could pave the way for the creation of more effective antimicrobial treatments and the optimisation of biofilm-dependent industrial processes.

B186

The Effect of Salt on Biofilm Formation by *Campylobacter jejuni* and *Campylobacter coli.*

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Abstract

Campylobacter jejuni and *Campylobacter coli* are commensal organisms of poultry and the leading cause of bacterial foodborne illness in humans. Biofilms formed by *Campylobacter* on poultry provide protection from control methods to prevent their spread, leading to increased human consumption. Previous research has shown that environmental stressors, such as salt and ethanol, can increase biofilm formation in other foodborne pathogens. The aim of this study was to determine the role of salt in biofilm formation in *Campylobacter* species. A panel of *C. jejuni* and *C. coli* isolates from different sources (lab, broiler, human, supermarket) were assessed for their ability to form biofilms at different temperatures in the presence and absence of salt by crystal violet staining. No differences were observed between isolates in the absence of salt but in the presence of salt, supermarket isolates showed higher levels of biofilm formation at lower temperatures. Immunofluorescent staining of biofilms in the presence of salt at 37℃ showed high levels of biofilm were decreased by salt while low levels of biofilm were increased by salt. Salt adapted mutants were generated by passaging isolates on increasing levels of salt up to 1.25%. Biofilm formation by salt adapted mutants in the presence and absence of salt was assessed and demonstrated a decrease in biofilm formation in the absence of salt compared to the wild type strains. In conclusion, the presence of salt can act as an inducer of biofilm formation in *Campylobacter* species but is dependent on both isolate source and environmental conditions.

B188

Deciphering the roles of mitochondria in Candida albicans biofilm formation: a novel approach therapeutic development

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Abstract

Candida albicans biofilm formation poses a formidable challenge in clinical settings, particularly in immunocompromised individuals fitted with medical devices. As biofilms are known for their treatment resistance and the establishment of persistent infections we require new and innovative approaches for effective intervention. We aim to determine the roles that mitochondria play in biofilm formation as a route to establishing as a new approach to treatment.

To achieve this we are making use of respiration inhibitors and mutant strains to map mitochondrial functions to biofilms specific traits that are relevant to their pathogenic and drug resistant traits. We have also establish a chemical biology pipeline that has led to the development of new and fungal specific mitochondrial inhibitors as potential therapeutics.

This multi-faceted approach seeks to unveil the intricate interplay between mitochondrial dynamics and *C. albicans* biofilm formation, providing insights into the potential of mitochondria as a viable drug target.

B189

The role of polymicrobial biofilms in urinary tract infections (UTIs) using a urine tolerant human urothelium organoid model (3D-UHU)

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Abstract

Urinary tract infections (UTIs) are among the most frequent types of infection (Öztürk and Murt, 2020). The global incidence of UTIs in 2019 reached 0.4 billion cases, with an associated increased mortality (Zeng, 2022). UTIs are also a major driver of the century's biggest challenge: antimicrobial resistance (AMR). The role of biofilm formation in UTI is poorly understood and is mainly focused on catheter infections. The possibility of biofilm-like communities on the bladder surface of non-catheterised UTI patients might explain the treatment failure and the high recurrence rates. As a related issue, polymicrobial biofilms are present in almost all types of human chronic infections. However, polymicrobial infection is challenging to study in vitro and tend to be understudied, especially in the UTI field (Armbruster, 2021).

Herein, we investigated the biofilm-forming capabilities of diverse uropathogens in human urine-containing media instead of standard culture media. Additionally, we evaluated the fitness of paired combinations of the most prevalent UTI pathogens in human urine vs standard media. Unexpectedly, certain pathogen combinations coexist in standard media but exhibit competitive dynamics in urine-containing media. Furthermore, we explored the biofilm formation of uropathogens as a single species and as dual combinations, employing a state-of-the-art 3D urine-tolerant human urothelial (3D-UHU) microtissue model. Our study presents the first example of dual-species and three-species biofilms cultivated in the context of a complex human urothelium. These findings enhance our comprehension of single-species and polymicrobial biofilm dynamics in UTIs and underscore the significance of media selection and microenvironment in discerning pathogen interactions.

B190

Exploring Microbial Dynamics in Biofilms associated with Catheter-Associated Urinary Tract Infections (CAUTIs)

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Abstract

Catheter-associated urinary tract infections (CAUTIs) persist as a prominent concern in both community and hospital care settings, impacting approximately 50% of hospitalized patients with indwelling catheters. Hospital-acquired infections, of which 40% are identified as CAUTIs, underscore the urgency of addressing this issue. While previous research has predominantly focused on bacterial pathogens associated with urinary tract infections (UTIs), particularly Escherichia coli as the primary CAUTI uropathogen, the presence of other microorganisms on catheter surfaces remains a relatively underexplored territory.

Notably, Candida albicans emerges as the second most prevalent CAUTI uropathogen, yet our understanding of its association with CAUTIs remains limited. My aim is to investigate the intricate interplay between *E. coli* and *C. albicans* within biofilms formed under a stable and controlled environment designed to closely emulate human urine. By simulating conditions that mirror a catheterized bladder system, we aim to provide a nuanced understanding of the microbial dynamics crucial for devising effective treatments. This comprehensive exploration not only contributes to the fundamental knowledge of CAUTIs but also lays the groundwork for the development of targeted therapies aimed at either treating or preventing these infections.

B191

Identifying, categorising and exploiting *Mycobacterium tuberculosis* biofilm-derived phenotypes using a luciferase reporter biofilm model for novel drug discovery

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Abstract

Mycobacterium tuberculosis (*M.tb*) is the causative agent of tuberculosis (TB). TB is the leading cause of death from a bacterial infectious disease. The World Health Organisation estimated that 10.6 million people fell ill with TB in 2021 and 1.6 million people died from active disease. Lengthy and toxic drug therapies are hampering efforts to control this disease, as is the emergence of antibiotic drug resistance. Therefore, the introduction of new drug regimens using novel drugs is fundamental to eradicating this disease.

M.tb has been observed to grow as aggregated clumps or clusters of bacilli both *in vitro* and in lung tissue, as a single organism biofilm. This aggregated biofilm-like growth causes changes to the phenotype of the bacilli, inducing phenotypic heterogeneity that may impact antimicrobial drug efficacy. Here, we describe the development of an *M.tb* biofilm model to mimic aggregated extracellular growth in lung lesions and to measure drug action using a luciferase reporter system alongside 16s RNA and CFU. As expected, biofilm-derived *M.tb* exhibited tolerance to isoniazid, additional first line drugs including rifampicin, ethambutol and bedaquilline were also investigated. There were differences in drug tolerance observed between different media conditions, such as a decrease in pH and supplementation with cholesterol. *M.tb* biofilms will be further characterised by confocal microscopy and electron microscopy, and *M.tb*-derived populations investigated by flow cytometry and RNA profiling.

Identifying and characterising drug-tolerant *M.tb* populations will improve our understanding of the action of drugs *in vivo* and may help advance novel treatment-shortening drug regimens for TB.

B192

Biofilm biomass and matrix composition of *Pseudomonas aeruginosa* grown in three different host-mimicking media

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Abstract

Pseudomonas aeruginosa relies on its virulence factors and biofilm formation to persist in different infection sites such as on medical implants, in wound burns, and lungs of people with cystic fibrosis (CF), which makes them resistant to antibiotics and host immunity. Generally, standard *in vitro* media routinely used in the laboratory do not meet the nutritional cues that influence the biofilm development at several infection sites. In this study, three host-mimicking media were used to assess the biofilm biomass and composition of *P. aeruginosa* PA14. These were synthetic wound fluid (SWF), synthetic cystic fibrosis medium (SCFM), and synthetic airway surface liquid (ASL) which mimics wound fluid, airway fluid of intubated patients, and lungs of people with cystic fibrosis respectively. The biofilm biomass was assessed through crystal violet staining. Matrix compositions of the biofilm were assessed through enzymatic treatment with DNase, proteinase K, and glycoside hydrolases (GH). PA14 showed observable colour difference in the different media. The biofilm biomass of *P. aeruginosa* PA14 varied in different host-mimicking media. Varied amount of eDNA and proteins were observed in biofilm grown only in SCFM and ASL as indicated by the activity of proteinase K ($P < 0.01$) and DNase ($P < 0.05$). Polysaccharide was prevalent in the biofilm formed in all the media. These demonstrate that *P. aeruginosa* can modulate its biofilm formation in response to nutrient that mimic infection sites within the human host, and this can help inform more targeted approach to treatment.

B194

Conditions for biofilm formation in extrapathogenic E. coli (ExPEC)

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Abstract

Sepsis is a complex multi-organ state that manifests itself as a dysfunctional immune response to infection. Recent estimates suggest that there are 48.9 million cases of sepsis worldwide resulting in 11 million deaths accounting for 20% of all deaths worldwide. In the same year in the UK, 245,000 cases of sepsis were estimated to cost the NHS directly up to £1.1 billion. Furthermore, the economic burden is even greater with up to £10 billion in societal costs. Bacteria cause at least 62% of sepsis cases, and extrapathogenic *Escherichia coli* (ExPEC) are a major contributor locally in Wales and the Hywel Dda University Health Board. ExPEC has many virulence factors (VF) that they utilise to evade the immune system, but less work has focused on their use of biofilm as a virulence mechanism. This work used biofilm and growth curve assays, confocal imaging and related patient phenotypic data to determine the conditions necessary for biofilm formation in these novel ExPEC bacteraemia isolates from the Hywel Dda Health Board. Our data suggests that iron, bile salts and mechanical stress are essential stimuli for biofilm formation in ExPEC. These results confirm that biofilm formation is a vital mechanism for ExPEC to survive in their local origin of infection, before translocation into the blood. This work will aid in understanding ExPEC pathogenesis and contribute to risk stratification to predict severe infections.

B195

Novel 3D co-culture model to investigate oral dysbiosis and resistome.

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Abstract

The rise of antimicrobial resistance (AMR) poses a significant threat to modern healthcare. Recent estimates suggest a projected 10 million annual deaths attributed to AMR infections by 2050. To effectively combat AMR, it is crucial to identify and understand the reservoirs and transmission routes of antimicrobial resistance genes (ARGs). Human commensals including members of oral microbiota, are considered important reservoirs of ARGs. While the presence of ARGs in the oral microbiota is well-documented, the intricate relationship between ARGs and oral dysbiosis remains largely unexplored.

To address this knowledge gap, we have developed a novel 3D co-culture model, using buoyant epithelial culture devices (BECD) integrating oral epithelial DOK/hTERT gingival fibroblast cells grown in collagen gels with complex *in vitro* biofilms. This model supports the longitudinal tracking of taxonomic and functional shifts from a healthy to a diseased state (typical of caries and periodontitis conditions) using DNA/mRNA sequence data (Illumina). We aim to investigate the evolution of the oral resistome from dysbiosis onset, and following dysbiosis reversal strategies.

Initial *in silico* analysis revealed significantly enriched ARGs (such as *ErmF* and *pgpB*) between the oral resistome of healthy and diseased individuals ($padj<0.05$, $Log2FoldChange>1$) and significant correlations between disease-associated genera and clinically relevant ARGs including *Porphyromonas gingivalis* with *ErmF* ($p>0.005$).

Our novel 3D co-culture model provides a valuable tool for understanding the progression of oral dysbiosis to disease and its role in the development of AMR, with great potential to contribute to the prevention and management of oral and systemic health issues.

B196

Photodynamic killing activity of *Pseudomonas aeruginosa* and MRSA in artificial sputum medium biofilm model

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Abstract

Persistent, long term bacterial colonisation and infection of the lungs are a major problem in patients with cystic fibrosis (CF), with difficulty to clear production of thick and viscous sputum in the airways that promotes the formation of bacterial biofilms which are often resistant to antibiotic treatment. Antimicrobial photodynamic therapy is a promising approach to eradicate antibiotic resistant bacteria via the bactericidal activity of reactive oxygen species produced by the combination of visible light, oxygen and a photosensitiser.

We investigated the photokilling anti-bacterial activity of blue light in *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms using an in vitro artificial sputum medium (ASM) model mimicking the sputum found in the CF lung environment. We also assessed the susceptibility of biofilms to antibiotics following light exposure and whether pre-sensitisation of biofilms with a photosensitiser could enhance light activity.

We found that blue light had a significant photokilling activity on both *P. aeruginosa* and MRSA biofilms in ASM and altered the growth of remaining viable bacteria leading to a cell growth delay. Following light exposure, biofilms were more susceptible to antibiotic treatment as they displayed a greater reduction in CFU counts. Moreover, a pre-sensitisation of biofilms with a photosensitiser in addition to the blue light exposure enhanced the antibiotics activity on biofilms.

Our results showed that blue light has significant bactericidal activity on CF-like biofilms, alters bacterial growth, and improves the antibiotics activity on biofilms, an effect that is enhanced when biofilms are treated with a photosensitiser.

B198

Biofilm disruption through controlled antimicrobial release from ultrasound-responsive silica nanoparticles

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Abstract

Root canal infections are difficult to treat, as bacteria colonise the microscopic network of dental tubules within the tooth, forming biofilms. Considering the ever-increasing antimicrobial resistance, it is imperative to utilise methods that are targeted, allowing for treatment at the site of infection, ideally without disrupting the surrounding healthy microbiome. Silica nanoparticles encapsulating suitable antimicrobials, which are only released upon a trigger, can be used as delivery vehicles into the tooth structure due to their ease of chemical synthesis, tuneability, and biocompatibility. Here, we present new designs of silica nanoparticles containing antimicrobial agents for the treatment of *Staphylococcus aureus* biofilms *in vitro*. We also introduce fluorescent agents onto the nanoparticle to track delivery and biofilm penetration. Novel silica nanoparticles have been synthesised using a one-pot method for encapsulating drugs and fluorophores. Particle size was measured with dynamic light scattering and transmission electron microscopy, and loading of the particles was estimated with UV-Vis spectroscopy and thermogravimetric analysis. Release upon ultrasound has been monitored. Biofilms on coverslips were treated with the ultrasound-triggered particles.Viability and penetration were measured using live/dead staining, confocal microscopy, and colony counting. Treatment with ultrasound-triggered particles showed significantly more dead bacteria than controls, showing a synergistic effect of ultrasound and triggering drug release from the nanoparticles. Nanoparticle penetration into deeper layers of the biofilm after the application of ultrasound was confirmed. In summary, antimicrobials can be released from silica nanoparticles using ultrasound as the trigger, which leads to triggered killing and stimuli-responsive drug delivery system for treating biofilm.

B199

Exploring the biofilm breaking potential of artificial sweeteners

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Abstract

Antimicrobial resistance (AMR) is recognised by the World Health Organisation (WHO), as one of the most pressing concerns of our time. Over-prescription and incorrect usage of antibiotics have further contributed to the worsening crisis. It is now widely recognised that we are in urgent need of novel therapeutic intervention strategies to tackle the wave of multidrug resistant infections sweeping through out healthcare systems. This urgent need to identify new compounds with antibiotic properties has prompted scientists to explore new reservoirs to identify potential therapeutics. One reservoir that is emerging with significant therapeutic potential is dietary compounds such as artificial sweeteners. Here, we show that artificial sweeteners including acesulfame K (ace-K) and saccharin can limit the growth of clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Both of these pathogens are notorious biofilm formers and adopting this mode of growth is seen as central to their pathogenic success. We demonstrate that artificial sweeteners can inhibit biofilm formation in both of these species, but also that they can disrupt pre-established biofilms. Clinically, many of these pathogens are rarely found in single species biofilms but as part of polymicrobial biofilm communities. We show that both ace-K and saccharin can prevent polymicrobial biofilm formation and disrupt established polymicrobial biofilms comprised of *P. aeruginosa, A. baumannii* and *Staphylococcus aureus*. Lastly, we demonstrate that these sweeteners can be successfully loaded into hydrogel wound dressings and limit infection progression in an *ex vivo* porcine burn wound model.

B200

Catching an ESKAPEd Pathogen: Investigating the Synergistic Application of Antimicrobial Peptoids against *P. aeruginosa*

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Abstract

Pseudomonas aeruginosa is a human pathogen for which there is an urgent need for novel treatment options. The list of effective antibiotics is becoming shorter and shorter as this pathogen has diversified its strategies of evading their activity. *P. aeruginosa's* ability to form biofilms in order to escape antibiotics is especially harmful to cystic fibrosis patients. This evasion can lead to chronic and reoccurring infections. We aim to overcome these evasion strategies by using novel antimicrobials "peptoids" in combination with current antibiotics to resensitise the bacteria. Checkerboard assays were used to determine which antibiotic-peptoid combinations are synergistic. These also provide initial guidance on the optimal concentrations required to treat *P. aeruginosa* infections. Antibiotic-peptoid combinations that were deemed synergistic, included antibiotics that *P. aeruginosa* displays intrinsic resistance to and are, therefore, not usually used to treat such infections. Synergistic combinations also included those antibiotics that, due to their toxicity, are used sparingly. When combining these antibiotics with our peptoids their therapeutic window could be lowered to nontoxic concentrations. Using air-liquid interface cultures, we will apply these combinations under physiologically relevant conditions to treat *P. aeruginosa* biofilms.

Furthermore, understanding how these combinations exert their superiority over conventional treatment will lead to more treatment options against *P. aeruginosa* infections. This will help to individualise drug regimes as a precision medicine approach by identifying the best therapeutic combinations for various antibiotic resistant strains.

B201

Characterising strain-level genetic and phenotypic diversity of Staphylococcus aureus in Chronic Wound Biofilms

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Abstract

Staphylococcus aureus is a pathogenic opportunist and one of the most frequently isolated organisms from diabetic foot ulcers and wound infections. *S. aureus* infection and persistence is mediated by the production of various virulence factors, and biofilm formation, which aids in host-immune evasion and development of resistance to systemic antibiotics. *S. aureus* strains exhibit a high degree of genetic diversity, with certain strains linked to worse healing outcomes (e.g. SA10757). Understanding this strain-level diversity is important for developing effective therapies that selectively inhibit *S. aureus* during chronic infections. Here, we isolated clinically relevant *S. aureus* strains from various chronic wounds and assessed strain-level diversity through long-read whole genome sequencing and phenotypic characterisation of antimicrobial resistance and biofilm-forming capabilities. Additionally, we identified other species capable of forming strong biofilms *in vitro*, such as *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. We reveal notable genetic and phenotypic diversity in *S. aureus* isolated from chronic wounds, which correlated with clinical outcome measures. Interestingly, *P. aeruginosa* and *S. aureus* were co-isolated from late-stage chronic wounds*,* exhibiting higher levels of resistance to most antibiotic classes screened. Thus, our data show the heterogeneity of *S. aureus* isolates and reveal that co-infection with *P. aeruginosa* substantially increases *S. aureus* tolerance to antibiotics, which has major relevance for polymicrobial infection management in the clinic. In addition, our recent work demonstrated the selectivity and efficacy of a novel endolysin against a subset of *S. aureus* strains*.* Our future work will therefore validate the effectiveness of this innovative, phage-extracted endolysin on clinical *S. aureus* isolates.

B202

To stay or not to stay: the challenge faced by immigrant AMR species entering handwashing sink drains

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Abstract

Handwashing lies at the forefront of infection control and prevention in healthcare, yet multiple studies have implicated water-based devices as reservoirs for the spread of resistant organisms. Despite routine disinfection, biofilms form on the luminal surfaces of drains and pipes, creating high density microbial aggregates that can be dispersed during faucet use. There is a paucity of knowledge about how immigrant species, bacterial species that integrate into existing local communities, establish and persist. The aim of this project was to develop a model system of the sink drain to study the impact of different immigrant AMR species on biofilm formation and microbial community interactions.

A continuous culture model of the sink drain was developed using the CDC biofilm reactor. Organisms were grown in the presence of different pipe materials and synthetic hospital wastewater (SHWW) to simulate conditions found in sink drains. Synthetic communities comprising of four common sink-dwelling genera were generated. Preliminary findings indicated that SHWW supported the growth of AMR species. Of the three pipe materials tested, biofilm formation was higher on chlorinated polyvinyl chloride (CPVC) rather than high-density polyethylene (HDPE) or polypropylene (PP).

Future work will focus on interactions between the synthetic community and immigrant AMR species, and examine the spatial architecture of these populations using fluorescent microscopy techniques. Once completed, this project should contribute to our understanding of sink-associated biofilms and their role in AMR dissemination.

B203

Functional and regulatory characterisation of a novel *Pseudomonas aeruginosa* (PA) secretory protein that is enriched in the biofilm matrix and its adjacent secretory system.

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Abstract

Pseudomonas aeruginosa (PA) is a prolific secretor of proteins and a model organism for studying biofilm formation. The biofilm matrix plays a key role in this by protecting the cells that it encases from chemical and physical attack. Secreted proteins must also pass through the matrix to pass into the wider environment. This raises the question of whether secreted proteins are selectively retained in the matrix? That is, is the matrix associated with a distinct "matrixome", and if so, how does this affect the properties of the biofilm.

Proteomic analysis revealed that one abundant matrix-associated secreted protein is PA2668, a cysteine-rich protein that bears little similarity to any previously characterised proteins. PA2668 is encoded immediately downstream of an uncharacterised Type II secretion system (T2SS), designated as the *hpl* gene cluster. The substrates of many T2SS are encoded next to their preferred secretion system, so it is likely that PA2668 may be exported through the Hpl system. To investigate the role of PA2668 and Hpl, we have begun a detailed characterisation of what regulates their expression. *LacZ* fusions, DNA pulldowns and protein detection using single chain antibodies (scFv) are being used for this. Data indicates that *hpl* expression is under quorum sensing control.

B206

Repurposing of Thioridazine as an efflux pump inhibitor against *Proteus mirabillis* catheter associated urinary tract infections (CAUTI)

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Abstract

Proteus mirabilis forms extensive crystalline biofilms of urinary catheters that block urine flow and complicate the care of catheterised patients. Previous work has highlighted the importance of efflux systems to *P. mirabilis* biofilm formation, and the potential to control catheter blockage through inhibition of these pumps using existing drugs from the SSRI and thioxanthene families. In this study we screen a range of available drugs from these families for potential efflux pump inhibition (EPI) in *P. mirabilis* and show the relative fluorescence of ethidium bromide is significantly greater in the treated samples than untreated. This demonstrates the EPI activity.

To evaluate the impact of drugs with potential EPI activity on catheter blockage we employed an in vitro model of the catheterised urinary tract, and measured the impact of Thioridazine (TDZ) treatment on time taken for catheters to block and the formation of biofilms. In addition, catheter encrustation was visualised using scanning electron microscopy (SEM). These experiments demonstrated that TDZ at 400 mg mL^{-1} significantly increases the time needed for *P. mirabillis* block catheters, and SEM visualisation indicated this was particularly related to the occlusion of catheter eye-holes by crystalline material. However, quantification of overall biofilm formation using a modified crystal violet assay showed no significant difference in biofilm formation on catheters from TDZ treated vs control models. Collectively these data suggest that Thioridazine can significantly delay catheter blockage and may specifically interfere with aspects of biofilm mineralisation.

BLOCK B

Session : Infection Forum (Block B)

B207

Metabolic profiling of airway infection environments identified branched chain amino acids as signatures of bacterial colonisation

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Abstract

Streptococcus pneumoniae is a leading cause of community-acquired pneumonia and bacteraemia and is capable of remarkable phenotypic plasticity, responding rapidly to environmental change. Pneumococcus is a nasopharyngeal commensal, but is responsible for severe, acute infections following dissemination within-host. Pneumococcus is adept at utilising host resources, but the airways are compartmentalised and those resources are not evenly distributed. Challenges and opportunities in metabolite acquisition within different airway niches may contribute to the commensal-pathogen switch when pneumococcus moves from nasopharynx into lungs. We used NMR to characterise the metabolic landscape of the mouse airways, in health and during infection. Using paired nasopharynx and lung samples from naïve animals, we identified fundamental differences in metabolite bioavailability between airway niches. Pneumococcal pneumonia was associated with rapid and dramatic shifts in the lung metabolic environment, whilst nasopharyngeal carriage led to only modest change in upper airway metabolite profiles. NMR spectra derived from the nasopharynx of mice infected with closely-related pneumococcal strains that differ in their colonisation potential could be distinguished from one another using multivariate dimensionality reduction methods. The resulting models highlighted that increased branched-chain amino acid (BCAA) bioavailability in nasopharynx is a feature of infection with the high colonisation potential strain. Subsequent analysis revealed increased expression of BCAA transport genes and increased intracellular concentrations of BCAA in that same strain. Movement from upper to lower airway environments is associated with shifting challenges in metabolic resource allocation for pneumococci. Efficient biosynthesis, liberation or acquisition of BCAA is a feature of adaptation to nasopharyngeal colonisation.

B208

Narrow spectrum antibiotics for the prevention and treatment of soft-rot plant disease

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Abstract

Worldwide losses to plant diseases are conservatively estimated at approximately US $150 bn, of which about one third are attributable to bacterial infections. In many cases, good sources of resistance for breeding are not available and the chemicals used to prevent spoilage are increasingly deemed environmentally unacceptable. The use of broad-spectrum antibiotics in the food chain is also undesirable as this can lead to the selection of antibiotic resistant strains of bacteria that may ultimately cause infections in humans. Bacteriocins are potent naturally produced protein antibiotics that have a narrow-killing spectrum and could be deployed to target a specific pathogen while leaving the wider plant and soil associated microbiomes intact. Soft-rot plant disease is caused through the infection of plants by bacteria such as *Pectobacterium* spp. and can occur in a range of economically important crops such as potatoes.

Our current work focusses on the identification, production and testing of novel bacteriocins targeting *Pectobacterium* spp. We will use a range of bioinformatic, genomic and biochemical tools to determine the mechanism of action of identified *Pectobacterium* targeting bacteriocins and test the efficacy of bacteriocins in soft-rot plant disease models.

B212

Phenotypic characterisation of the effect of fluoroquinolone resistance on *Campylobacter jejuni*

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Abstract

Campylobacter jejuni is the leading cause of bacterial gastroenteritis and is commonly found on poultry meat. The World Health Organisation has designated *C. jejuni* as a high priority pathogen due to rising levels of antibiotic resistance and, in particular, resistance to fluoroquinolone antibiotics. This rising level of antibiotic resistance occurs despite efforts to regulate and reduce the use of antibiotics in the poultry industry. Recent studies have reported a correlation between fluoroquinolone resistance and aerotolerance in *C. jejuni*. This could give resistant bacteria an advantage in transmission through the food chain, where exposure to oxygen is inevitable. In this study, fluoroquinolone resistant mutants of laboratory and fresh isolates of *C. jejuni* were generated through repeated passaging on agar plates supplemented with increasing concentrations of ciprofloxacin. As a result, a panel of isolates with low (up to 10µg/mL), medium (up to 40µg/mL), and high levels (>40µg/mL) of ciprofloxacin resistance were obtained for each strain, and PCR was used to confirm the presence or absence of mutations in the *gyrA* gene, which are associated with ciprofloxacin resistance in *C. jejuni.* The susceptible and resistant variants were analysed for changes in multiple phenotypes including aerotolerant growth, motility and biofilm formation.

B213

Development of a porcine genome-wide CRISPR/Cas9 screen to identify host dependency factors for swine influenza virus

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Abstract

Swine influenza virus has a significant financial impact in pig breeding, while simultaneously raising alarms over catastrophic pandemics due to its zoonotic nature. As an obligate intracellular parasite, it relies on host factors for its replication. Understanding of host-pathogen interactions is required to identify novel therapeutic targets and further design disease control. High throughput phenotypic screens can be a powerful tool in achieving this with CRISPR/Cas9 allowing systematic analysis of mammalian genomes. Here we developed a genome-wide CRISPR/Cas9 knockout screen in porcine cells infected with influenza A virus (IAV), in order to identify host dependency factors. Infected cells were sorted based on the level of IAV infection, and the functional relevance of genes enriched in each population was assessed by the quantification of sgRNAs through the MAGeCK pipeline. Several genes previously identified as essential for influenza infection, such as SLC35A1, WDR7 and ATP6AP1 were also identified as important for IAV replication in our screen, giving us confidence that the approach was successful. In addition, many novel genes were identified as IAV host dependency factors. Currently, validation of these genes and the effect of their disruption on IAV replication is ongoing. These studies will provide a genome wide analysis of virus host interactions for swine influenza virus in porcine cells and the basis for additional characterisation and comparative studies with influenza A screens in human and chicken cells.

B214

Investigation of in-host adaptation of sequential clinical Mycobacterium abscessus isolates to the cystic fibrosis lung environment

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Abstract

Chronic infection by opportunistic pathogens is a major contributor to mortality in people with cystic fibrosis (CF). *Mycobacterium abscessus* is an emerging pathogen of concern in CF, causing recalcitrant infections with high antibiotic resistance. *M. abscessus* adapts over time of colonisation to conditions in the CF lung. The mechanisms underlying this pathoadaptation are poorly understood and could be key for future therapies. In this study, four pairs of longitudinal sequential clinical isolates of *M. abscessus* spanning 33 and 783 days from people with CF attending Saint Vincent's University Hospital were investigated.

TimsTof Mass spectrometry identified proteomic changes associated with pathoadaptation in late isolates. Interestingly, there were 21 proteins identified which were absent in early isolates but highly abundant in late isolates including: Bifunctional AAC/APH (aminoglycoside resistance), MnmA (intramacrophage survival), AhpD (antioxidant defence), FadE18 (cholesterol catabolism) and LipC (immune modulation). Heparin-binding hemagglutinin (HbhA) which is involved in epithelial cell attachment was also increased 3.5-fold. Confirmatory phenotypic analysis showed altered colony morphotype, increased biofilm production (up to 8-fold, $p \leq 0.01$) and increased sliding motility (up to 5-fold, $p \leq 0.001$). Consistent with HbhA alterations, attachment to human lung cells was increased by up to 2-fold. Late isolates also showed altered antibiotic resistance, including a significant increase in resistance to azithromycin ($p \leq 0.001$).

These findings indicate that *M. abscessus* undergoes adaptation to the CF lung environment in as little as 33 days. Better understanding the mechanisms underlying adaptation is crucial for developing novel therapeutics to treat and eradicate this difficult pathogen.

B215

Historical differences between strains of *Acinetobacter baumannii* result in consequential differences in tigecycline response and resistance evolution

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Abstract

Evolutionary history encompasses genetic and phenotypic bacterial differences, but the extent to which history influences drug response and antimicrobial resistance (AMR) adaptation is unclear. Historical contingencies arise when elements from a bacterium's past leave lasting effects on the genome, constraining paths available for adaptation.

To study this, we utilized reference strains of *A. baumannii* exhibiting broad historical differences: a laboratory strain, 17978-UN, and a clinical strain, AB5075-UW, and imposed stress using the last-resort antibiotic, tigecycline (TGC). RNA sequencing of the strains grown under subinhibitory TGC revealed diverging transcriptional responses suggesting that pre-existing transcript levels may dictate gene expression in response to drug stress.

To test how evolutionary history influences AMR evolution, we propagated both strains in TGC. Whole population sequencing revealed that population dynamics differed between strains. However, AMR was obtained through predictable mechanisms of increasing drug efflux and target modification. Despite a shared efflux pump repertoire, we saw strong strain-specific efflux preferences. AB5075-UW acquired a variety of mutations to the *adeRS-ABC* efflux operon while 17978-UN acquired mutations with site-specific parallelism to *adeL*, the regulator for the *adeFGH* efflux system. AMR adaptation is often associated with a fitness tradeoff, in our study, this phenomenon was only seen in 17978-UN lineages.

This work shows that even under strong antibiotic selection, the influence of history on adaptation prevails. In evolving populations, history reduces genomic and phenotypic predictability between strains. Understanding the predictability of AMR evolution will enable efficient treatment strategies and help to halt the spread of multi-drug resistant organisms.

B216

Examination into antibiotic resistance mechanisms employed by clinical isolates of *Pseudomonas aeruginosa* from Cystic Fibrosis patients in Irish Hospitals.

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Abstract

Pseudomonas aeruginosa is a nosocomial pathogen infecting cystic fibrosis patients and capable of adapting to its environment and evolving to overcome the stresses of host immune systems and antibiotics (1). Antibiotic resistance in *P. aeruginosa* is a major challenge in treatment of infection and drug resistant *P. aeruginosa* is listed as a Priority Pathogen by the World Health Organisation (WHO) (2). Clinical respiratory isolates (n=8) were obtained from patients with cystic fibrosis and examined using Kirby-Bauer disk-diffusion and broth microdilution methods to determine their antimicrobial susceptibility profiles. Using Clinical Laboratory Standards Institute (CLSI) (3) guidelines, multidrug-resistant (MDR) isolates (n=2) were identified from this group, and further testing employed for potential antibiotic resistance mechanisms. Biofilm forming capabilities were tested using crystal-violet staining method (4) and these isolates were found to be poor biofilm formers. Efflux-based resistance mechanisms were examined by permeability assays such as the Cartwheel Method (5) and real-time fluorometric methods (6), both using ethidium bromide dye. The two MDR clinical isolates showed a relatively high permeability level when examined by these methods, indicating high efflux capability. Given the multitude of efflux pumps encoded in the *P. aeruginosa genome* (7, 8) the antibiotic resistance displayed by these clinical isolates is likely mediated by efflux pumps and mechanisms related to bacterial cellular permeability. This provides an insight into the mechanisms of resistance circulating in current *P. aeruginosa* infections in cystic fibrosis patients and suggests that therapeutics targeting efflux pump function may be strong candidates as alternative methods for treating antibiotic resistant infections.

B217

Development of a Smart Diagnostic Platform to Simultaneously Identify Viral Respiratory Illnesses

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Abstract

Viral respiratory tract infections represent a worldwide public health problem. Influenza viruses result in more than 32 million lower respiratory tract infection cases in adults yearly. The recent global pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has also highlighted the need for reliable, accurate, and affordable molecular diagnostic assays to differentiate between multiple respiratory viruses. In recent decades, Reverse transcription loop-mediated isothermal amplification (RT-LAMP) has proven effective as a nucleic acid amplification test compatible with point-of-care testing (POCT). RT-LAMP reaction can be done using fluorescent real-time detection, which increases the sensitivity and functionality of this method.

Our research focuses on developing a simple, cost-effective, sensitive, and specific real-time fluorescent RT-LAMP POCT system that can detect 7 respiratory viruses simultaneously in less than an hour.

We are developing two panels of 13 respiratory viruses, including influenza A, influenza B, parainfluenza 1-4, respiratory syncytial virus (RSV) A, RSV B, SARS-CoV-2, human metapneumovirus, adenovirus, rhinovirus, and bocavirus. The reaction is conducted in a small portable device wirelessly linked to a smartphone. To simplify the sample preparation process further, we designed a proprietary hand-held small closed system module to facilitate our novel target enrichment and extraction process, prevent sample contamination, and provide robust on-site sample processing.

Developing an efficient comprehensive low-cost POCT system that can detect different respiratory viruses with a simple on-site sample preparation step will improve viral surveillance and diagnosis. This will be a great breakthrough in molecular diagnostics, especially in resource-limited circumstances such as low income countries.

B218

Determining the mechanism of killing of the *P. aeruginosa* targeting bacteriocin pyocin AP41

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Abstract

Gram-negative pathogens such as *P. aeruginosa* are common causes of nosocomial infections such as ventilator-associated pneumonia, which are increasingly difficult to treat due to the spread of antimicrobial resistance. Bacteriocins, which are narrow-spectrum protein antibiotics, are a potential alternative to the small molecule broad-spectrum antibiotics currently used in clinical practice. One such bacteriocin is pyocin AP41, which unlike other now better characterised bacteriocins in its class, is poorly understood, in terms of its mechanism of targeting and killing *P. aeruginosa*. Elucidation of its mechanism of action could enable engineering of the protein into an effective therapy.

We have shown that pyocin AP41 is highly effective in killing a diverse range of *P. aeruginosa* isolates and is highly effective in infection models. The aim of our current work is to define the mechanism through which pyocin AP41 is able to target *P. aeruginosa* and transport its DNase cytotoxic domain across the cell envelope into the cytoplasm where it cleaves genomic DNA. To achieve this, we will isolate pyocin AP41 resistant mutants and use whole genome sequencing to identify potential receptors and/or transporters parasitized by this pyocin. We will then use a combination of genetic and biochemical approaches to confirm interactions of candidate cell envelope proteins with pyocin AP41 and define their role in targeting and uptake.

B219

Manuka Honey as a Treatment for Chronic Cystic Fibrosis Lung Infection.

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Abstract

Background: *Pseudomonas aeruginosa* is a multidrug resistant (MDR) opportunistic pathogen, which can cause chronic lung infection in people with cystic fibrosis and lead to potential respiratory failure and complications due to host inflammatory response. To address this, we have examined whether manuka honey can be used alone or in combination with anti-pseudomonal antibiotics to reduce bacterial load and moderate inflammatory response in the upper and lower airway.

Methods: A murine model with an established chronic *P. aeruginosa* lung infection and uninfected control was established. Manuka honey (30%), tobramycin and manuka/tobramycin combination was administered via intranasal saline. The effect of these treatments on bacterial load and inflammatory markers such as IL-6, TNF-α was evaluated via total viable counts and ELISA.

Results: The total viable counts (CFU/ml) 24 hours post treatment were significantly reduced (p=<0.05) in the nasopharynx and lungs in the manuka honey and tobramycin combination group compared to control.

Both IL-6 and TNF-α were significantly reduced (p=<0.05) by combination treatment in the lungs but not in the nasopharynx. Indicating the combination treatment of manuka honey and tobramycin causes less inflammation in lung tissue than treatment with manuka honey and tobramycin alone.

Conclusion: These results show that in combination with antibiotics the manuka honey actively reduces viable infection and inflammation in chronic *P. aeruginosa* lung infection and could be considered as a potential future therapeutic for these infections.

B220

A sweet tooth: Does twitching motility in the absence of sugar determine tumour invasion by gut streptococci?

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Abstract

Streptococcus gallolyticus subspecies *gallolyticus* (*Sgg*) is an opportunistic Gram-positive gut pathogen, long associated with colorectal cancer (CRC). *Sgg* translocates into the bloodstream via cancerous gut lesions causing bacteraemia and infective endocarditis in CRC patients. Closely related gut streptococci, *S. pasteurianus* and *S. lutetiensis*, also cause bloodstream infections but are not associated with CRC.

The aim of this study is to compare the behaviour of *Sgg,* and related species in the presence/absence of sugars. Having recently shown all three species display twitching motility, we hypothesised that differences in twitching motility and subsequent colorectal epithelial cell invasiveness may explain the association of *Sgg* with CRC that closely related species lack.

For motility assays, streptococcal bloodstream isolates were inoculated on Todd Hewitt agar with/without glucose (0.5%). Following incubation for 48 h, colony diameters were measured. Streptococci grown with glucose had a significantly smaller colony diameter than controls ($p<0.0001$), while *Sp* is more motile in the presence of glucose than *Sgg* ($p=0.029$).

Invasion assays consisted of incubating HT-29 colon cells with streptococci grown in the presence/absence of glucose (0.5%) for 1 h, followed by gentamicin treatment, serial dilution, and plating. Glucose reduced the ability of streptococci to invade colon cells, however, *Sp* and *Sl* are more invasive than *Sgg* in the presence of glucose ($p<0.0001$).

Glucose reduces the motility and invasiveness of all three species, however, *Sgg* appears to be more affected by glucose than both other species. This could help to explain why *Sgg* is more associated with CRC than *Sp* and *Sl*.

B221

Ruthenium metallotherapeutic agents: novel approaches to combatting *Pseudomonas aeruginosa*.

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Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen which is highly resistant to antibiotics and biocidal products. There is an ongoing need to develop novel approaches for combatting antimicrobial resistant infections caused by *P. aeruginosa*. Metals have been used as antimicrobial agents throughout history for a broad range of applications. Ruthenium (Ru) metallotherapeutic compounds have potent antimicrobial properties and in contrast to traditional antibiotics, these are thought to elicit antibacterial activity at multiple sites within the bacterial cell, thereby reducing the possibility of resistance evolution. Minimum inhibitory and bactericidal concentration (MIC / MBC) assays, coupled with disc diffusion assays were used to screen a library of Ru metallotherapeutics. One lead compound was identified which was highly active at inhibiting growth of multiple clinical strains of *P. aeruginosa* at ≤32 µg mL^{-1}, with loss of viability occurring within 6 h. Crystal violet biofilm assays showed a decrease in biomass following exposure of *P. aeruginosa* biofilms over a 24 h period. Scanning electron microscopy was used to reveal morphological changes in the bacterial cell ultrastructure after exposure, with evidence of membrane perturbation which supported a proposed mechanism of antimicrobial activity. Cell culture *in vitro* scratch assays and 3D skin full thickness wound infection modelling were used to demonstrate the wound healing potential of the lead Ru metallotherapeutic. These findings make a significant contribution towards the search for novel bactericidal agents and further research is now focussed on determining the potential for use as novel adjuvants within medicinal applications.

B222

Long-term hypoxia promotes adaptations in *Pseudomonas aeruginosa* consistent with adaptations observed during chronic infection.

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterial pathogen which causes chronic lung infections in people with cystic fibrosis (CF) and is a major contributor to morbidity and mortality. Despite this, the mechanism driving its adaptation towards chronic colonisation in the CF lung is not yet fully understood. Hypoxia is one of the important environmental pressures present in the CF lung. This work focuses on the adaptations of *P. aeruginosa* to long-term hypoxia to investigate whether it drives the development of persistence in CF patients.

We studied the effect of long-term adaptation of an early CF isolate to 6% oxygen for 28 days. Two distinctive small colony variants (SCVs) developed, one SCV exclusively under low-oxygen pressure. Importantly, SCVs were more common in hypoxia-adapted cultures, comprising up to 98% of the population, while never exceeding 35% in normoxia-adapted cultures. Proteomic analysis showed significant changes in the abundance of >200 proteins within 28 days, including those involved in antibiotic resistance, stress response and biofilm formation. Two hypoxia-adapted cultures developed higher resistance to 8 out of 13 antibiotics tested and showed increased biofilm (4.08-fold and 1.80-fold (pV<0.0001)) and exopolysaccharide production. The third population displayed resistance to only two antibiotics and showed decreased biofilm-forming capability. All hypoxia-adapted cultures developed higher resistance to osmotic stress, while two hypoxia-adapted populations also showed increased resistance to oxidative stress but decreased resistance to high temperature.

This confirms that hypoxia promotes the development of phenotypes associated with persistence in the lung and poor patient outcomes in *P. aeruginosa.*

B223

Deciphering the pathogenic and immune evasion strategies of *Streptococcus gordonii*, a bacterial endocarditis pathogen

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Abstract

Streptococcus gordonii, a VGS oral commensal is an important causative organism of native valve infective endocarditis (IE). *S. gordonii*'s adaptations include PadA and Hsa, and SsnA, a DNAase. PadA and Hsa have been extensively studied, but their roles in blood survival are yet to be fully described. SsnA homologues digest NETs *in vitro*, but the ability of SsnA to modulate neutrophil behaviour is not yet understood. This study compared *S. gordonii* WT with isogenic and double mutants to determine these adaptations' importance to (1) blood survival, (2) host protein binding and (3) neutrophil modulation. (1) Broth-cultured bacterial cells were incubated in blood, with viable counts at two and five hours. Survival was significantly reduced with the ablation of either or both PadA and Hsa. Overnight incubation of strains in serum to induce 'serum adaptation' – phenotypic change resulting in accumulation of peptidoglycan, masking both adhesins and bound opsonins – improved survival of mutant strains to levels comparable with WT after five hours' blood exposure. (2) WT, Δ*padA*, Δ*hsa* and Δ*padA*/Δ*hsa* bound complement inhibitors FH and Vn, and coagulation protein Fn, though additional study will determine any differential binding between strains. (3) Neutrophil ROS response to broth-cultured WT, Δ*padA*, Δ*hsa*, Δ*padA*/Δ*hsa* – was measured over four hours. Δ*hsa*, Δ*padA*/Δ*hsa* induced significantly less ROS; however, serum adaptation both reduced total ROS response and eliminated any significant difference between strains. These data further demonstrate the importance of PadA and Hsa as *S. gordonii* virulence factors, supporting their investigation as treatment targets for IE.

B224

Exploring the Secretion of TslA, a novel 'reverse' toxin of the *Staphylococcus aureus* Type VII Secretion System

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Abstract

The type VII secretion system (T7SS) is a protein export pathway that is found in many Gram-positive bacteria and in mycobacteria. To date, all substrates of the T7SS substrates share a common architecture - the N-terminal domain forms a helix-turn-helix domain (often containing a WXG or LXG motif), that is required for secretion and dimerisation, with a C-terminal functional domain of variable length. Excitingly, we have identified a completely new family of T7SS substrates that shows the reverse structural organisation; with the helical secretion domain instead at the C-terminus. We have characterised a member of this new family, TslA, from *Staphylococcus aureus*. TslA has a phospholipase domain at its N-terminus. Two small proteins encoded at the *tslA* locus interact with TslA and facilitate its secretion by the T7SS, acting as small partner proteins for TslA. Motifs like those mentioned above are also possessed by TslA and it's small partner proteins, however, the motifs themselves appear to also be reversed (such as a GXW motif instead of a WXG motif). Using NanoLuc Binary Technology, secretion of TslA through the T7SS was measured using NanoLuc assays involving fusion of pep86 (the small subunit of a luciferase protein) to TslA. New data will be presented on the impact of mutating single amino acid residues in either TslA or the two small partner proteins, on the secretion of TslA via the T7SS.

B226

Mammalian glycosyltransferase inhibitors as new *Legionella* specific antimicrobials

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Abstract

Legionnaires' disease is a severe type of pneumonia caused by the Gram-negative facultative intracellular pathogen *Legionella pneumophila*; an environmental bacterium found in natural and man-made water systems, capable of infecting human alveolar macrophages. Once internalised, the bacteria weaponize the Dot/Icm Type IV secretion system to translocate a large arsenal of effectors to actively manipulate host signalling mechanisms enabling bacterial survival and replication. Glycosyltransferase effectors are a key component of this arsenal; prompting us to hypothesize that the manipulation of host protein glycosylation is an important part of the infection strategy and could be a target for new antimicrobials.

Using continous fluorescence-based intracellular and absorbance-based planktonic broth growth microplate assays we identified two inhibitors that, while designed and marketed for a mammalian glycosyltransferase, had unexpected direct activity against *L. pneumophila.* Mechanistic studies showed a significant decrease of ATP levels in the bacteria and increased cell envelope permeability, suggesting that enzymes critical for cell envelope synthesis might be inhibited. Determination of the activity spectrum showed inhibition of *Legionella* species, but not *E. coli* and a selection of other Gram-negative pathogens. Taken together, our work suggests that *Legionella* spp. possess a unique Achilles' heel and identified candidate drugs for repurposing or as lead molecules to exploit this weakness for developing new, selective antibiotics for Legionnaires' disease.

B227

Discovery and Functional Annotation of Type VI Secretion Systems in *Legionella* Species

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Abstract

Ubiquitous in nature, the environmental pathogen *Legionella* employs its Dot/Icm Type IV Secretion System (T4SS) and more than 300 effectors to enter and replicate within phagocytes. The T4SS proteins DotU and IcmF share homology with structural components of Type VI Secretion Systems (T6SS); versatile nanomachines widely distributed among Gram-negative bacteria used to deliver effectors directly into neighbouring bacterial and/or eukaryotic cells using a phage-like piercing mechanism. However, while a few other T6SS-related genes have been reported, it remained unclear if *Legionella* species encode complete T6SS.

This study used a variety of bioinformatic programs to systematically analyse the distribution and integrity of T6SS clusters across the *Legionella* genus. This identified that 10 species encode complete clusters and highlighted structural and organisational differences. Prototypical isolates of *Legionella pneumophila*, the primary human pathogen of the genus, do not contain T6SSs. Expression analysis of *Legionella birminghamensis* revealed components of this system are upregulated in early exponential growth phase. While sharing little sequence similarity with characterised Hcp proteins, Alphafold modelling suggests *Legionella* Hcp-proteins do not contain effector domains and form characteristic hexameric Hcp rings that form the T6SS tube. Structure and sequence homology analysis of the VgrG proteins revealed additional extensions without similarity to characterized proteins, indicating that these VgrG proteins might have evolved new effector-domains and/or effector-loading mechanisms.

Our data suggests that T6SSs are part of the arsenal of several *Legionella* species, which could represent so far underappreciated driver of the ecology and virulence of these environmental and opportunistic human pathogens.

B228

Skin Manifestations of Geriatric Patients with Chikungunya Encephalopathy

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Abstract

INTRODUCTION

Chikungunya belongs to togaviradae family, which causes plethora of skin manifestations including maculopapular rash, vesicles and bullae, dermatitis like rash. Cutaneous lesions affect trunk, limbs and face which may be pruritic. We report 4 patients of chikungunya encephalopathy with only over, altered sensorium as presenting complaints, but could be diagnosed as a case of chikungunya on the basis of different skin features.

METHODS

All the cases of chikungunya encephalopathy were followed up from the data collected in the Department of Internal Medicine and Neurology at Sir Gangaram Hospital, over a period of 6 months from May 2023 to November 2023. The case files of the patients with encephalopathy were studied.

RESULTS

We reported 4 cases of chikungunya encephalopathy. Among these all patients were male with an age above 60 years. Fever was the presenting complaint in all 4 (100%) patients. Joint pain was only present in 1 (25%) patient. Skin manifestations in the form of maculopapular rash were present in 2 (50%), skin blistering in 1 (25%) and bullae in 1 (25%) patient. All 6 (100%) cases, received steroids in the form of dexamethasone. Severe lung infection developed in 1 (20%) patient. 4 (100%) patients were discharged in a stable condition.

CONCLUSION

Currently there are no specific antivirals or vaccines against this virus and hence remains a challenge for clinicians to treat. Not all patients present with typical features, particularly geriatric population , so skin manifestations might help in clinching the diagnosis.

B230

The probability of resistance cell establishment is influenced by antibiotic dose and interaction with the sensitive population.

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Abstract

Designing effective treatment strategies is crucial in reducing antimicrobial resistance (AMR) burden. A robust understanding of how antibiotic exposure impacts evolution of AMR is needed. An essential step in this process is proliferation of a resistant cell to establish a resistant population. Previous work showed that antibiotic concentrations well below the minimum inhibitory concentration (MIC) are sufficient to reduce probability of establishment. However, under specific antibiotic concentrations a sensitive population provides a protective effect to the resistant cell, significantly increasing establishment probability. Work presented here builds upon these findings. Our aim is to understand whether this phenomenon is seen across multiple antibiotics and resistance mechanisms or specific to the strains and antibiotics studied. While the previous study only focused on strains expressing resistance genes from a plasmid, this work has also used strains that evolved resistance under antibiotic pressure. Our results confirm that antibiotic concentrations below MIC are sufficient to reduce establishment probability, but the strength of this effect varies between antibiotics. The protective effect of a sensitive population has been observed for two streptomycin resistance mechanisms but is not seen with ciprofloxacin treatment, where presence of a sensitive population only negatively effects resistant cell establishment. Ongoing work aims to understand the mechanisms of protection by measuring antibiotic sequestration by sensitive cells and studying effects of secreted cell products on resistant cell establishment. This work furthers our understanding of the establishment of a resistant population and helps inform selection of antibiotic concentrations best able to restrict AMR evolution.

B232

Development of a WHO International Standard for anti-Marburg Virus Antibodies

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Abstract

Marburg virus (MARV) causes Marburg virus disease, a severe illness with a case fatality rate of up to 88%. There is currently no licensed vaccine against MARV, but several candidates have entered clinical development and a number of immunological assays have been developed. To increase comparability of results generated by these assays, an International Standard (IS) is required. The IS is the highest order of reference reagent for biological substances and established by the WHO Expert Committee on Biological Standardization (ECBS). The quantification of sample potency using assays calibrated against the IS is reported in a common unitage, which facilitates comparison between laboratories.

To develop the first WHO IS for anti-MARV antibodies, sera from eight healthy survivors of the 2012 MARV outbreak in Western Uganda were sourced and tested for binding and neutralisation activity using anti-MARV glycoprotein (GP) ELISA and VSV based pseudotyped virus neutralisation assays, respectively. The neutralisation activity was assessed against two MARV GP (Ci67 and Musoke) and a Ravn virus GP (Kitum Cave). Testing was performed in parallel at the MHRA and Battelle to increase confidence in sample characterisation. Individual sera showed anti-GP IgG binding activity of various levels, from undetectable to 7582 ELU/mL. Neutralisation activity was low for all samples, ranging from undetectable to 75 ND50, and varied depending on the strain. A candidate IS was prepared by pooling the sera presenting the highest binding activities and lyophilised. The results from a large ongoing multi-centre collaborative study evaluating the candidate IS will also be presented.

B233

Development of sustainable, antimicrobial essential oil microcapsules for use within healthcare textiles.

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Abstract

Current antimicrobial coatings for textiles are often toxic and non-environmentally friendly. Microcapsules have many potential medical uses, for example drug delivery and diagnostics. This project investigates encapsulating an antimicrobial natural product blend of *Litsea cubeba* and *Citrus limon* essential oils in a sustainable, non-toxic shell of sodium alginate polymer gel for use against the healthcare-associated pathogens *Staphylococcus aureus, S. epidermidis, S, capitis, Escherichia coli,* and *Pseudomonas aeruginosa.*

This study aims to investigate a sustainable, environmentally friendly alternative to current antimicrobial finishes for healthcare textiles.

A 1:2 blend of *Litsea cubeba* and *Citrus limon* achieves antimicrobial efficacy against *S. aureus, S. epidermidis, S, capitis, E. coli* and *P. aeruginosa*. Preliminary investigations of the natural blend show inhibition of bacterial growth ranging within 15-30 minutes in solution. Disc diffusion, minimum inhibitory concentrations and time-kill assays were utilised to determine antimicrobial efficacy of the free and encapsulated natural product blend.

A novel, smart microfluidic control system (*Fluigent, France*) was used to standardise microcapsule materials, size (95-160µm) and production rate (1200 mc/hr). Microencapsulation of the essential oil blend has been achieved with a concentration of 40µl/ml in each core, inhibiting the growth of the tested microorganisms. Determination of the microcapsule release profile is underway by investigating the release of the active core using pressure (texture analyser, *TA-XT Plus*) and diffusion over time.

The use of a microfluidic microencapsulation process for natural products has shown promising results as antimicrobials, further investigation of microcapsule distribution and antimicrobial efficacy within textiles is required.

B234

Investigating the evolution of resistance in *Staphylococcus aureus* and *Pseudomonas aeruginosa* to Balds eyesalve and derived cocktail.

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Abstract

Antimicrobial resistance (AMR) in bacteria is a growing global public health concern with many bacterial infections becoming increasingly difficult to treat with current conventional antibiotics. Without intervention, by the year 2050 it is predicted that there will be 50 million deaths a year resulting from AMR. One promising avenues for antibacterials that we can turn to are "ancientbiotics". Bald's eyesalve is one of these antibacterial remedies used originally in the 10th century to treat eye infections. It is derived from the combination of natural ingredients including wine, garlic, bile salts and another *Allium* species. Research over the last 8 years within the Harrison Lab at the University of Warwick has shown that Bald's eyesalve and its derivatives have potent antibacterial and antibiofilm activity and can serve as alternatives for treating bacterial infections. However, further characterisation of potential evolution mechanisms to the remedies is required before the treatments can progress further in trials. A long-term resistance evolution assay exposing bacterial pathogens to sub-inhibitory concentrations of these remedies in cation adjusted Mueller-Hinton broth (caMHB) and synthetic wound fluid (SWF) shows that organisms take longer to develop resistance against these remedies compared to conventional antibiotics. In conclusion, Bald's eyesalve and derived cocktail could be better alternatives for treating bacterial infections than current conventional antibiotics. Additionally, they can also be combined with conventional antibiotics to reduce the drive for antimicrobial resistance evolution.

B235

Prevalence of Shiga Toxin-Producing *Escherichia coli* O157 in Scottish Farmed Deer

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Abstract

Shiga toxin-producing *E. coli* (STEC) are zoonotic pathogens associated with infection in humans globally, which, in severe cases, can be life-threatening. STEC serogroup O157 (STEC O157) are responsible for most human cases in the UK with Scotland having the highest incidence (4-5 per 100,000). Livestock, particularly cattle, are the primary reservoir of STEC O157 and infection in humans is considered incidental via contact with contaminated food or water. The importance of other wildlife species, such as Deer, in STEC O157 transmission from livestock to humans has been highlighted in recent studies.

This pilot study aimed to assess the presence of STEC O157 in farmed deer, where the stocking density is significantly higher than that of wild deer. 361 deer faecal samples were collected from 10 individual deer farms across Scotland. Single colonies of STEC O157 were isolated following selection on CT-SMAC agar and STEC O157 positive colonies were confirmed by latex agglutination test.

STEC O157 was confirmed in deer faecal samples on 6/10 farms tested and 31 samples were found to be positive for STEC O157. The presence of virulence genes *stx1 stx2* and *eae* was confirmed by PCR and STEC O157 isolates were whole genome sequenced to further characterize their virulence gene profiles.

Previously, we determined that the prevalence of STEC O157 in wild Scottish deer was low (0.28 %). In contrast, significantly higher levels of STEC O157 were detected within the farmed deer populations assessed (8.59%), consistent with published data in cattle.

B236

It's not over yet: the ocular complications of leprosy in post-elimination India

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Abstract

Over half of the world's MB leprosy cases are in India. In 2005, India's government declared leprosy elimination status and integrated specialised leprosy services with general healthcare.

India's integrated leprosy healthcare has left leprosy patients vulnerable to ocular disability and other morbidities.

Multi-drug therapy does not treat or halt the progression of ocular complications hence 1-2% of patients go on to develop cataracts, lagophthalmos and uveitis. In the post-elimination era, cooperation is needed between NGOs and primary to tertiary level healthcare services to manage ocular manifestations in leprosy patients.

Unfortunately, the influence of stigma cannot be understated as leprosy patients are less likely to access healthcare services due to the lack of specialist leprosy care delivered at general medical health centres, geographical distance, financial capability and societal ostracism.

Local healthcare infrastructure and socioeconomics influenced how integration was implemented across states with poorer areas recording lower levels of specialist healthcare training and resource allocation.

This scoping review was conducted over a 8-week period. Peer-reviewed quantitative and qualitative papers were found using MEDLINE (Ovid). Ethical approval was not obtained because primary data was not used.

In conclusion, leprosy-related ocular disability has been unaddressed by India's integration strategy. The lack of communication between tiered healthcare services, reduced emphasis in healthcare training and access to specialist services result in greater difficulty for identifying and diagnosing ocular complications of leprosy in post-elimination India. In future, early detection of ocular complications could reduce the level of disability in leprosy patients.

B237

Targeting bacterial G-quadruplex DNA with small molecule ligands leads to promising antibacterial activity against *Escherichia coli*

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Abstract

With the current issues of bacterial antimicrobial resistance (AMR), there is great need for the development of novel antimicrobials to tackle infections caused in particular by Gram-negative species such as *Escherichia coli*. Here we demonstrate that a novel pyridinium-functionalised azobenzene scaffold L9, identified from a screen of G-quadruplex (G4) ligand candidates, is a promising agent with antibacterial activity (MIC values ≤ 4 mg/ml) against multi-drug resistant (MDR) *E. coli*. Tandem Mass Tag (TMT) proteomics of *E. coli* treated with sub-inhibitory concentrations of L9, identified G4-associated open reading frames as potential targets for L9. Biophysical analyses (fluorescence resonance energy transfer (FRET), circular dichroism (CD) and UV-visible spectroscopy) was conducted and confirmed that L9 binds to these selected sequences with variable affinity, compared to the two comparator G4 ligands (stiff-stilbene L5 and pyridostatin (PDS)) that better stabilise G4s but have lower antimicrobial activity. High-resolution fluorescence microscopy-based Bacterial Cytological Profiling (BCP) suggests that L9 mechanism of action is distinct from currently prescribed antibiotics representing classes that inhibit DNA replication, RNA transcription, protein translation and membrane integrity. Next-generation sequencing and X-ray crystallography experiments are underway to further identify and characterise the mode of G4 binding by L9. These findings support further exploration of G4 ligands as potential novel therapeutic agents with G4-mediated mechanisms of action.

B238

Exploring the Poly-γ-DL-Glutamic Acid (PGA) Layer in Coagulase-Negative Staphylococci as a Promising Target for Anti-Virulence Therapy

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Abstract

Staphylococcus epidermidis is a Gram-positive opportunistic pathogen that forms a poly-γ-dl-glutamic acid (PGA) layer. PGA acts as a defense against the host immune system and antimicrobial agents, however, its contribution to biofilm formation remains poorly studied. We aimed to better understand the role of PGA in virulence by bioinformatically identifying other staphylococcal species capable of PGA production and to develop an anti-PGA enzyme-based therapy.

We screened 3,783 high-quality *Staphylococcus* genomes for four PGA biosynthesis genes and identified 11 coagulase-negative *Staphylococcus* (CoNS) species harbouring all genes with >90% coverage and >70% identity to our PGA positive reference. Many of the species identified cause infections in immunocompromised individuals, making them promising targets for an anti-PGA therapy.

Next, we cloned, expressed, and purified a PGA depolymerase (EnvD) to determine its therapeutic potential. To ascertain if depolymerase treatment could restore sensitivity to antibiotics, resistant CoNS isolates were exposed to EnvD and differences in MICs measured. Furthermore, we examined if the hydrolysis of PGA could contribute to the prevention and disruption of biofilms using the crystal violet assay. Preliminary results indicate that depolymerase treatment reduces biofilms, suggesting PGA has a role in biofilm formation and/or maintenance.

Lastly, we investigated the role of PGA in a systemic infection model using *Galleria mellonella*. Larvae were infected with either wild type *S. epidermidis* or a PGA negative mutant. We observed no significant differences in morbidity or mortality over 72 hours, suggesting the role of PGA in virulence is nuanced and that more relevant infection models are required.

B239

Combatting antimicrobial resistance: Small-molecule demethylase inhibitors as precision antimicrobial and anti-virulence agents

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Abstract

Antimicrobial resistance (AMR) is a growing global public health issue, and it is estimated that deaths associated with AMR infections will exceed 10 million by 2050. Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a priority pathogen for the development of treatment strategies. Using small-molecule inhibitors (SMIs) designed against epigenetic bacterial processes, including methylation, represents an innovative approach to combatting AMR by modulating key bacterial virulence traits including biofilm formation and toxin production. Antimicrobial susceptibility screening identified one lead SMI that exhibited activity at <6.25 µM against a range of clinical MRSA strains. A 76% reduction in MRSA biofilm formation was observed in cells treated at sub-inhibitory levels, along with a 23% reduction in haemolytic activity. Gene expression studies using qRT-PCR against MRSA exposed to sub-lethal SMI concentrations revealed upregulation (6.46-fold) in *sasG* which is involved in the accumulation phase of biofilm formation. Despite the observed increase in gene expression, phenotypic biofilm formation was significantly reduced after exposure to the SMI which suggested the anti-biofilm effect cannot be recovered. No changes in *icaB* expression were observed, suggesting that the SMI disrupted biofilm formation at the accumulation phase rather than initial colonisation. At biologically relevant concentrations, no cytotoxicity against mammalian skin cell lines including NHDF was observed. Research is now focused on validating the SMI bacterial target and identifying global SMI-mediated proteomic changes to further elucidate the role of methylation. This research represents a major advance in the search for novel antimicrobial agents which target essential bacterial processes beyond those associated with traditional antibiotics.

B240

The artificial sweetener saccharin disrupts bacterial membrane stability and DNA replication dynamics

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Abstract

Antimicrobial resistance is one of the most pressing concerns of our time. Deaths caused by it and its burden to global healthcare systems are increasing at an alarming rate, resulting in the antibiotic resistance crisis. One possibility to develop novel antimicrobial therapies is to identify and repurpose compounds with antimicrobial activity whose safety for humans is well established, such as artificial sweeteners (AS). In previous work, we demonstrated that saccharin, which is among the most extensively used AS world-wide, impacts the growth of pathogens including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* by an unknown mechanism. Here, we demonstrate that saccharin inhibits growth via DNA damage in an *Escherichia coli* model. This damage activates break-induced replication systems, accumulating replication foci in the chromosome, thus generating genome instability. As an additional antimicrobial mechanism, saccharin impacts cell morphology and produces bulge-mediated cell lysis. Interestingly, the antimicrobial activity of saccharin could be reproduced on different clinically relevant pathogens, including the World Health Organisation top-priority carbapenem-resistant *A. baumannii*, thus suggesting a broad-spectrum activity. Saccharin treatment also affected the *A. baumannii* membrane stability, impacting the cell morphology and producing bulge-mediated cell lysis. Furthermore, using fluorescently-labelled penicillin probing, we show that saccharin increases membrane permeability. This resulted in a resensitisation to the last-resort antibiotics carbapenems. Lastly, we show that saccharin can successfully be included in hydrogel formulations to treat burn wounds in an *ex vivo* pig skin model. Altogether, our findings provide compelling evidence supporting ASs, as saccharin, as promising antimicrobial candidates or antibiotic potentiators against multidrug-resistant pathogens.

B241

Identification and characterisation of vaccine antigens for Klebsiella pneumoniae

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Abstract

Klebsiella pneumoniae is a Gram–negative bacterium, which causes septicaemia, respiratory tract infections, urinary tract infections and soft tissue infections. It is a major opportunistic pathogen, accounting for 11.9 % of all reported cases in the EU/EEA in 2021, and is also a typical antimicrobial resistant pathogen, associating with high mortality rates.

This project aims to identify proteins used by *K. pneumoniae* to attach to host epithelial cells and their potential as novel protective vaccine antigens. In total, 31 bacterial adhesins from *K. pneumoniae* were identified by the Cell Blot approach developed by the McClean Lab, 24 of which were novel. Four antigens were selected as potential vaccine candidates. These were confirmed to play a role in *K. pneumoniae* attachment to lung cells *in vitro*, as BL21 cells expressing recombinant proteins showed 14.3–, 7.22–, 6.48–, and 13.3–fold (p=0.012, p=0.0199, p=0.0011, p=0.045[SM1]) increased levels of attachment to 16HBE14o^{-} cells respectively. Immunisation of mice with Antigens L, D or O individually showed reduced *K. pneumoniae* burden in peritoneal cavities (1.54 log, 2.69 log, 2.50 log (p=0.0080, p=0.0007, p=0.0007), respectively) in a sepsis challenge model and also reduced dissemination to the spleen (by up to 1.4 log_{10}). Serological analysis showed the expression of high antigen–specific antibody responses compared to the control group. T–cell recall response analysis following immunisation of Antigen L or O demonstrated stimulation of IL–17 and IL–22 expression. Overall, these antigens were protective against *K. pneumoniae* sepsis and have potential as vaccine candidates.

B242

Hypoxia drives alterations in *Burkholderia cenocepacia* that are consistent with adaptations during chronic colonisation of the cystic fibrosis lung.

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Abstract

Burkholderia cenocepacia is an opportunistic pathogen that causes chronic lung infections in people with cystic fibrosis (CF). *B. cenocepacia* possesses a multireplicon genome encoding a range of genes involved in adaptation to the CF lung. We previously reported that two series of *B. cenocepacia* chronic infection isolates showed an increased abundance of up to 149 proteins, including 19 proteins encoded in the low oxygen (Lxa) locus in both patients and increased attachment to human lung cells. We hypothesise that detection of, and response to, reduced oxygen concentration drives persistence of *B. cenocepacia* in the CF lung, ultimately leading to chronic infection. To investigate this, *B. cenocepacia* was exposed to either hypoxic (6% oxygen) or normoxic conditions for 22-days *in vitro*, with sampling every 2 days. Proteomic analysis revealed an increased abundance of proteins previously associated with the transition to chronic colonisation of the CF lung. In hypoxia-adapted isolates, zinc metalloproteases were increased in abundance 4-fold relative to normoxic isolates. Additionally, 7 proteins encoded on the Lxa locus were also significantly increased in abundance including a metallo β-lactamase (4.5-fold). Increased abundance of universal stress proteins (USPs), implicated in survival in the CF lung, was also observed. Hypoxia-adapted isolates showed increased protease activity ($p<0.001$), host cell attachment ($p<0.01$) and resistance to antibiotics ($p<0.05$). Overall, these data are consistent with the hypothesis that long-term exposure to hypoxia drives adaptation of *B. cenocepacia* to the CF lung and influences the expression of virulence factors associated with increased rates of morbidity and mortality.

B243

Filamentous structures consistent with hybrid viral particles form upon coinfection with IAV clinical isolate and RSV

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Abstract

Coinfections, involving the infection of a single host by multiple viruses play a crucial role in shaping transmission dynamics and clinical outcomes. A previous study from our lab, focusing on the viral interactions at the cellular level, described the formation of hybrid viral particles (HVPs) as a consequence of coinfection with laboratory-adapted strains of IAV and RSV, which exhibited altered antigenicity and receptor tropism. To delve into and understand the formation of hybrid viral particles further, we chose to infect A549 cells with a prototype strain of RSV and a clinical isolate of IAV H3N2 , which has been the predominant subtype during the last flu seasons and also allowing us to make use of a more biologically relevant system. Using fluorescence imaging, we observed filamentous structures, resembling hybrid viral particles and carrying surface glycoproteins from both IAV and RSV. Notably, these filamentous structures exhibited an altered staining profile, with considerably more HA present, compared to what was previously shown upon coinfection of cells with IAV and RSV prototype strains, suggesting variable implications about the antigenicity and tropism of these particles. Furthermore, initial findings have revealed that this clinical isolate of IAV is not able to spread efficiently in this cell line, and yet, during coinfection it was able to significantly reduce RSV infection, presumably through the interferon response. These initial findings bring us closer to answering the overarching question, which is if HVPs can happen in nature and investigate their potential implications on virus pathogenesis.

B245

Small-molecule kinase inhibitors as precision anti-virulence agents.

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Abstract

Antimicrobial resistance (AMR) is an ever-increasing global issue, and it is estimated that deaths associated with AMR infections will exceed 10 million by 2050, superseding cancer as the leading cause of global mortality. Traditional antibiotics target key bacterial processes such as cell wall formation which are essential for viability but susceptible to resistance evolution. In contrast, one approach to combatting AMR is the development of novel small-molecule inhibitors (SMIs) as precision anti-virulence agents, which target metabolic pathways, sporulation, heat shock responses, quorum sensing and virulence. This study aimed to elucidate the antimicrobial and anti-virulence activity of a novel series of SMIs which were rationally designed against protein kinases that are implicated in phosphorylation-dependent quorum sensing pathways. Antimicrobial screening identified lead hit candidate SMIs which demonstrated low MIC and MBC values against methicillin-resistant *Staphylococcus aureus* (MRSA) and were fast acting as determined by time-kill kinetic assays. The effect of SMIs on biofilm formation and eradication were studied using biomass staining and viability assays. At biologically relevant concentrations, these SMIs exhibited no cytotoxic activity against mammalian skin cell lines including Normal Human Dermal Fibroblasts. Research is now focused on elucidating the role of the SMIs in disrupting quorum sensing activity and the effect on *in vitro* virulence traits, in addition to validating the bacterial cellular target. This research represents a novel approach to developing the next generation of antimicrobial and anti-virulence agents.

B246

iPSC derived organoids as a model of cholera toxin

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Abstract

Cholera is an acute diarrhoeal disease caused by infection with the Gram-negative bacterium *Vibrio cholerae.* Infection occurs upon ingestion of *V. cholerae* via contaminated food or water, if left untreated can be life-threatening. The World Health Organisation estimates up to 4 million cases worldwide each year, with up to 143,000 deaths. The major virulence factor for pathogenic strains of *V. cholerae* is cholera toxin (CT), which is believed to illicit the profuse diarrhoea synonymous with cholera.

Historically, much of the cholera research field has been based upon animal models for bacterial colonisation and disease. Despite the wealth of research using animal models, the only known natural host of *V. cholerae*are humans. The main objective of this project is to add to the existing understanding of *V. cholerae* infection dynamics by developing a novel human model of infection using Human induced pluripotent stem cell (HuiPSC) derived intestinal organoids. All cell types present within the small intestine are represented in the culture, with complex architecture comprising of a basal lateral surface and an inner lumen. During the validation of the model, key similarities in how both animal models and the human intestine respond to CT were discovered. For example, we witness degranulation of goblet cells, leading to release of mucus that has been shown to be a response seen in rabbit ilium following exposure to CT. Furthermore, transcriptomic analysis reveals biomarkers linked to CT treatment which have previously been shown to be differentially regulated during natural infection in cholera patients.

B247

Novel Models for the Study of Host, Pathogen and Drug interactions in Cystic Fibrosis Lung Infections

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Abstract

Chronic pulmonary infection is the largest contributor to morbidity and mortality in people with cystic fibrosis (CF). Current antimicrobials have limited efficacy, and the development of novel antimicrobials is slow, as preclinical antimicrobial testing models poorly translate to the clinic. It is necessary to streamline the preclinical development pipeline, to develop models that reflect crucial factors of the CF environment and can be assessed for treatment-responsive, host-derived biomarkers associated with positive clinical outcomes.

This project aims to optimise *in vitro* and *in vivo* preclinical models that capture essential aspects of the CF host environment, including host factors and biomarkers that are associated with the resolution of airway exacerbation in a clinical setting. We are developing a cell culture infection model, whereby bronchial epithelial cells are infected with *P. aeruginosa* and treated with novel antimicrobials. Key biomarkers are then measured to predict clinical efficacy. We have also optimised a *Galleria mellonella* infection model. Greater wax moth larvae are a valuable prescreen to mammalian models and have an immune system similar to the human innate immune system. Larvae are infected with *P. aeruginosa*, and antimicrobials are subsequently administered at mg/kg equivalents of human dosages. Timing and dosage can be optimised prior to testing in more costly vertebrate models, while unpromising antimicrobials can be screened out.

Streamlining and standardisation of the preclinical antimicrobial development pipeline will increase the efficiency of product progression and facilitate collaboration between clinicians, industry, regulators and academics. This will ultimately improve clinical outcomes for those with CF.

B248

Postbiotic immunomodulation of chicken macrophages for the management of avian pathogenic *Escherichia coli* (APEC)

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Abstract

Avian pathogenic *Escherichia coli* (APEC) is the leading causative agent of poultry-associated bacterial disease. Historically managed using antibiotics but the emergence of resistance necessitates novel alternatives. Innate immunomodulation of poultry, such as immune priming or training, shows promise as a potential broad-spectrum control strategy. Here, *in vitro* tissue culture-based assays were utilised to determine the ability of the postbiotic butyrate to modulate macrophage responses to APEC.

Immortalised chicken macrophages (HD11) and chicken bone marrow-derived macrophages (BMDMs) underwent immune priming or training with butyrate prior to APEC challenge. Post-challenge, intracellular bacterial survival, macrophage viability, nitric oxide (NO) and reactive oxygen species (ROS) production were quantified. Phagocytotic impacts were determined using GFP-tagged *E. coli*. Furthermore, butyrate's mechanism of action was investigated using inhibitors of autophagy.

Butyrate priming and training of HD11 cells and BMDMs resulted in significantly reduced bacterial intracellular survival ($p \leq 0.05$), whilst maintaining macrophage viability. Phagocytotic capability was unaffected in primed BMDMs, but was significantly enhanced within primed HD11 cells ($p \leq 0.05$), suggesting reduced bacterial intracellular survival is attributable to improved bacterial killing. NO production was unchanged following butyrate treatment, while ROS production was significantly enhanced ($p \leq 0.05$). Interestingly, treatment of the cells with chloroquine, thereby blocking autophagosome fusion with lysosome and impeding lysosome acidification, attenuated butyrate-enhanced killing, suggesting a mechanistic role for autophagy.

Collectively, butyrate exposure improves chicken macrophage antimicrobial activity against multiple APEC lineages *in vitro,* potentially due to the enhancement of autophagy. This work highlights the prospect of targeting the innate immune system as a novel disease management strategy.

B249

Membrane proteins AgB and AgD, two potential antigens offering partial protection against *Acinetobacter baumannii* infections

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Abstract

Acinetobacter baumannii, a prominent cause of nosocomial infections, presents a formidable challenge due to its antibiotic resistance with limited therapeutic options. Our study spearheads vaccine development, employing the 'Cell Blot' approach for adhesin discovery and reverse vaccinology for vaccine shortlisting. This innovative platform entails probing bacterial membrane proteins with human bronchial epithelial (HBE) cells, detecting adherent cells with specific antibodies, followed by adhesin identification through LC-MS. Two adhesins, AgB and AgD emerged as promising candidates. Intriguingly, an *agB* knockout mutant exhibited a significant increase in adhesion to HBE cells (p=0.0023).

Full-length *agB* and *agD* genes were cloned and expressed in *ClearColi* (BL21), followed by Ni-NTA purification. Female C3H/HeN (6-8w) mice were subcutaneously immunised with 50 µg purified rAgB or rAgD with SAS adjuvant at D0, D14, and D28. Sera were collected on D35. Serological analysis revealed robust humoral responses, with total IgG, IgG1, IgG2a titres reaching 10^6, a ratio suggestive of a mixed Th1/Th2 response . On D42, mice were intratracheally infected with 10^7 CFU *A. baumannii* ATCC19606, establishing an acute mouse pneumonia model. At 24 hour post-infection, mice were sacrificed. Immunisation with rAgB significantly reduced lung bacterial burden by 0.87 $\log_{10}$ CFU (p=0.0098), while rAgD produced a 0.68 $\log_{10}$ reduction (p=0.0295) compared to the SAS-only group.

Overall, our study unveils the potential of two protective antigens, providing partial protection against *A. baumannii* in a mouse pneumonia model. These findings contribute critical insights to ongoing efforts in developing vaccines targeting multidrug-resistant *A. baumannii* strains, addressing a pivotal gap in combating emerging antibiotic resistance.

B250

Lateral flow as a novel approach for bartonellosis diagnosis

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Abstract

Bartonella are facultative intracelular Gram-negative bacteria with zoontic potential. The infections in humans range from mild with unspecific symptoms to potentially fatal, and they are mainly transmitted via blood-sucking arthropods. Worldwide, the most prevalent *Bartonella* species infecting humans is *Bartonella henselae*, a cat-related species. Although the first reports of human infection with *Bartonella* species date back to the early 1900s, diagnosis confirmation has proven to be extremely challenging. Currently, the IFA assay is widely used for diagnosis and in epidemiological studies. In order to improve the diagnose bartonellosis caused by *B. henselae*, we demonstrate here the performance of a novel lateral flow test utilizing recombinant protein along, with comparisons with ELISA and IFA assays. For that, serum samples from 64 cats (39 positive and 25 negative) were subjected to IFA, ELISA, and lateral flow for serological diagnosis of *Bartonella*. The *Bartonella* infection was confirmed using isolation on a chocolate agar plate and molecular methods (qPCR, PCR, and sequencing). Out of 39 positive cat-serum samples, 64% (25/39), 72% (28/39), and 82% (32/39) were positive when submitted to IFA, ELISA, and lateral flow assays, respectively. Sensitivity and especificity were 64% and 100% in the IFA, 71% and 88% in the ELISA, and 82% and 92% in the lateral flow, respectively. For IFA, ELISA, and lateral flow, the positive and negative predictive values were 100% and 64, 90% and 66%, and 94% and 76%, respectively. These findings demonstrate the lateral flow's potential as a screening tool for bartonellosis diagnosis.

B251

Macozinone Revealed: Nanomotion-Based Rapid Phenotypic Evaluation of New Drug Candidate for *Mycobacterium tuberculosis* Treatment.

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Abstract

The surge in global *Mycobacterium tuberculosis* (MTB) infections, compounded by the post-COVID-19 landscape, underscores the urgency for swift diagnostics, especially given the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Conventional antimicrobial susceptibility testing (AST), notorious for its time-intensive nature, impedes the prompt identification of drug-resistant cases, posing a challenge in managing escalating global MTB infections. This study delves into nanomotion-based AST, lauded for its ultra-rapid phenotypical capabilities in the 2023 Tuberculosis Diagnostics Pipeline Report (2) for its high performance on established antitubercular agents (RIF, INH) (1). Our focus lies in evaluating the adaptability of this method for detecting new drug candidate, such as Macozinone (MCZ).

Employing the Phenotech AST device, we assessed its efficacy in distinguishing Macozinone profiles, utilizing MTB susceptible (H37Rv) and resistant (NTB1 with DprE1 mutations) strains (3). Changes in bacterial metabolism patterns were correlated with drug susceptibility profiles, and a machine learning approach gauged the device's accuracy, sensitivity, and specificity in predicting strain phenotypes.

Susceptible MTB strains exhibited a notable decline in cantilever oscillations, signifying reduced bacterial metabolism and eventual inactivation. Conversely, resistant strains remained unaffected by the drug in their environment. Classification models for the DprE1 inhibitor Macozinone demonstrated high training accuracy, surpassing 95%.

The nanomotion-based rapid AST protocol was effectively applied to the developmental drug MCZ. Crucially, the approach validated Macozinone's novel effects *in vitro*. The Phenotech AST device exhibits promise for direct deployment in endemic countries, facilitating timely, accurate treatment decisions for patients with results delivered in under a day (21 hours).

B252

Mutation of the *chuA* haem receptor in *Campylobacter jejuni* increases intracellular survival in THP1 macrophages.

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Abstract

Campylobacter jejuni is a Gram-negative, microaerophilic bacterial coloniser of farmed animals and is a leading cause of human gastroenteritis. Iron is a crucial metabolite and co-factor for many processes that facilitate host gut colonisation. Over-coming the non-specific host defence mechanism of iron restriction is key. The ChuA receptor in *C. jejuni* is responsible for the uptake of haem bound iron. Our bioinformatic analysis of 18,784 *C. jejuni* and *C. coli* strains has shown the *chuABCDZ* operon is 100% conserved. Inactivation of the *chuA* gene in the *C. jejuni* NCTC 11168 strain had no effect on adhesion, invasion and survival within Caco-2 human intestinal epithelial cells or 8E11 chicken intestinal epithelial cells as compared to the wild-type strain. In contrast, a *chuA* mutation in the *C. jejuni* M1 strain led to a significant increase in adhesion, invasion and survival within Caco-2 cells and 8E11 cells. As *C. jejuni* can avoid trafficking to the lysosome in non-phagocytic cells but not phagocytic cells, we have examined survival of these mutants in THP1 differentiated macrophages. Both the M1 and NCTC 11168 *chuA* mutants showed significantly higher intracellular survival than the wild type and complementation strains. This improved survival of the mutants may be due to protection against the reactive oxygen species that play a key role in the host innate intracellular immunity. Hydrogen peroxide sensitivity assays have confirmed that M1 and NCTC 11168 *chuA* mutants show a decreased sensitivity compared to their wild-type counterparts.

B253

Rational design of an effective sub-unit vaccine against *Coxiella burnetii*

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Abstract

Ruminants, particularly sheep and goats, are the primary reservoir of *Coxiella burnetii* where infection can lead to abortions, still births and weak offspring. Vaccines are considered the most effective control against *C. burnetii* and killed whole cell vaccines based on virulent phase I strains are available. However, there are significant safety and manufacturing issues associated with current vaccines. **This study aimed to identify *C. burnetii* candidate vaccine antigens and design an effective subunit vaccine for use in livestock.**

To identify candidate vaccine antigens, peptide microarrays representing all *C. burnetii* ORFs were synthesized, and probed with sera generated from natural host species previously infected with or vaccinated against *C. burnetii*. In total, 493 seroreactive antigens were identified and the subcellular localisation was determined for each. Only antigens with outer membrane or extracellular loci were selected for vaccine development. The top six seroreactive antigens were formulated into two subunit vaccines and used to vaccinate mice in two independent *C. burnetii* challenge trials. Both subunit vaccines significantly reduced the splenomegaly phenotype in mice compared to unvaccinated controls. Compared with mice vaccinated using a commercial vaccine, Coxevac®, however, the subunit vaccines did not confer the same level of protection. Sheep, a natural host, were also immunised with each antigen and a strong antibody response was elicited against all six antigens.

Using reverse vaccinology, we have identified *C. burnetii* immuno-reactive antigens and demonstrated efficacy for two vaccine formulations. Additional candidate vaccine antigens are being investigated in combination with those used in vaccine formulations and potential diagnostic antigens have been identified.

B254

Assessing the efficiency of diverse mechanisms of horizontal gene transfer in clinical *E. coli* strains

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Abstract

Horizontal gene transfer (HGT) plays a crucial role in the dissemination of antibiotic resistance genes, contributing to the evolution of bacteria. *Escherichia coli*, as a significant opportunistic pathogen, has witnessed a rapid emergence of multidrug-resistant clones in recent years. We hypothesize that HGT may be involved in this process. However, the specific mechanisms of HGT that are most impactful in clinical *E. coli* isolates remain poorly understood. Therefore, in this study we assessed the efficiency of gene transfer mediated by various mobile genetic elements (MGEs), such as plasmids, phages, and phage-inducible chromosomal islands (PICIs), in a diverse panel of E. coli clinical strains. Subsequently, we investigated how different *E. coli* clones influence HGT. We utilised genomic information to identify bacterial immune systems that could potentially block the entry and/or incorporation of foreign DNA. Collectively, these results contribute to our understanding of how certain antibiotic-resistant strains rapidly emerge, becoming successful and dominant clones.

B255

Development of Novel Vaccines against *Acinetobacter baumannii* and *Pseudomonas aeruginosa.*

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Abstract

Acinetobacter baumannii and *Pseudomonas aeruginosa* are ESKAPE pathogens, classified as critical priority pathogens by the World Health Organization, and leading contributors to nosocomial infections. Despite the demonstrated success of bacterial vaccines in preventing infections and addressing antibiotic resistance, there are currently no approved vaccines for these pathogens.

We identified several novel adhesins as vaccine candidates, some of which are shared by both *P. aeruginosa* and *A. baumannii.* This research aims to examine the protective potential of a homologue, AgB, that is common to both species.

The homologues share 30.85% similarity and bioinformatic analysis predicted seven CD4 epitopes. The antigens were cloned, expressed, and purified for evaluation of their vaccine potential, with careful attention to minimizing endotoxin levels before preclinical studies in mice. *A. baumannii* AgB, adjuvanted with the Sigma adjuvant system was investigated for its protective capacity in immunisation and bacterial challenge studies in a mouse acute pneumonia model. The antigen was partially protective, reducing bacterial lung colonisation by 1.03-log_{10} ($p<0.0001$) and dissemination to the spleen by a 0.9-log_{10} reduction ($p<0.0426$). The immune responses will be evaluated using ELISA and flow cytometry.

The protective capacity of the *P. aeruginosa* AgB will undergo similar evaluation in mice. This research contributes to the urgent need for effective vaccines against antibiotic-resistant bacteria, presenting novel vaccine candidates against these two pathogens.

B256

Efflux, Signalling and Warfare in a Polymicrobial World

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Abstract

Efflux pumps are ancient transport systems that are ubiquitous in nature and play important, but poorly understood physiological roles in natural ecosystems, as the majority of the specific efflux pump signals/inducers remain to be characterized. They are best known for their role in antimicrobial resistance (AMR). The current Post-Antibiotic Era describes the rise in AMR/multi-drug resistance (MDR) mechanisms in pathogens and lack of novel antibiotic discovery. One such resistance mechanism is via efflux pumps.

Small molecule efflux pump inhibitors (EPIs) have gained considerable traction. EPIs can be viewed as an anti-infective strategy, rather than growth inhibition through enhancement of antibiotic activity. Efflux inhibition can impact cell-cell communication, quorum sensing (QS), vesicle production and other functionalities core to microbial physiology. The ability to block cell-cell communication, and thus, multicellular behaviour could suppress key virulence phenotypes such as biofilm formation and swarming motility.

One major challenge to the clinical development of EPI interventions is that microbes exist in diverse polymicrobial communities that undergo significant genotypic and phenotypic diversification. Off-target effects of EPIs on the diversity and stability of the community in the pathogen’s ecosystem must be considered in the context of lead molecule design and selection. A series of synthetic EPIs built upon the natural quinoline scaffold were studied for anti-virulence and anti-growth affects against various ESKAPE pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* in monoculture and mixed microbial populations. Different synthetic modifications of the same EPI chemical backbone were found to elicit a distinct response at the species level.

B257

Characterisation of a new family of *Legionella pneumophila* CE clan peptidase T4SS effectors

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Abstract

Infection of immunocompromised patients with *Legionella pneumophila* can result in a severe form of pneumonia, called Legionnaires' disease. The disease is a manifestation of the bacterium's ability to replicate intracellularly and manipulate cell signalling pathways in alveolar macrophages and lung epithelial cells. For this, *Legionella* translocates more than 330 effector proteins into the host cell cytoplasm via its Dot/Icm Type IVB secretion system. The function of most of these effector remains unknown.

We have identified a family of three T4SS effector proteins, which share structural but not sequence homology with CE Clan proteases. Mammalian CE clan protease typically remove ubiquitin-like modifiers from proteins to regulate cell signalling post-translationally; however bacterial CE clan protease effectors can have a wider substrate spectrum including ubiquitin and also be acetyltransferases.

Functional characterisation of the new effectors by ectopic-expression revealed that two of them localise to perinuclear vesicles whereas the third shows nuclear and cytoplasmic distribution. All three effectors do not have promiscuous deubiquitinase activity; however, the two vesicle-localised effectors induce an accumulation of ubiquitin and the autophagy marker LC3 in their proximity. Autophagy is a conserved eukaryotic pathway mediating protein turnover, nutrient recycling, and cytoplasmic infection control by entrapping ubiquitinated cargo or invading bacteria in a membrane-bound compartment for subsequent degradation. Our data suggest that *Legionella* co-opted the CE clan protease domain to evolve a functionally diverse effector family, whose membrane-targeted members manipulate central mechanisms of cellular homeostasis and cell intrinsic host defence.

B258

A vaccine for treatment or prevention of verotoxin-producing *Escherichia coli* (VTEC) infection.

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Abstract

Verotoxin-producing E. coli (VTEC) are a group of zoonotic foodborne pathogenic *E. coli* strains that are associated with causing bloody diarrhoea. VTEC infections can result in Haemolytic Ureamic Syndrome (HUS) which is the leading cause of kidney failure among young children. Ireland currently has the highest incidence of VTEC cases in Europe with 16.3 cases per 100,000. While antibiotic treatment is contradicted due to a large number of patients experiencing severe symptoms and complications, there is an unmet need for a vaccine to reduce mortality and reduce the greatest risk of kidney failure in children. As VTEC colonises gastrointestinal epithelial cells, bacterial adhesins involved in host-cell attachment represent promising vaccine candidates as previously shown to provide protective effects in other infections. We previously used proteomic approach to identify bacterial proteins involved in attachment to two human gastrointestinal epithelial cell lines, HT29 and Caco-2. Seven proteins in VTEC strain O157:H7, NCTC12900 were identified as novel adhesins, which were not found in commensal strain, HS. To date, one adhesin, GlnH, provided partial protection and reduced faecal shedding in immunised mice.

Four additional identified adhesins were selected for investigation as potential antigens including uncharacterized protein YiaF, Phosphoenolpyruvate-protein phosphotransferase (PPP), antigen A and antigen D for further analysis. To date, these antigens were found to be highly prevalent among the bovine and sheep isolates and have been successfully cloned, expressed, and purified for further evaluation in an immunised and oral challenge murine model.

B261

Identification of a Campylobacter jejuni metal efflux system that protects against reactive species generated by innate immune defences.

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Abstract

The Gram-negative bacterium Campylobacter jejuni causes many millions of cases of human gastroenteritis each year with infection typically acquired from consumption of poultry, particularly chicken. Campylobacter colonises the gastrointestinal tract and in humans this leads to significant inflammation and neutrophil influx with associated production of reactive oxygen species (ROS). Hence, Campylobacter has evolved to survive this oxidative stress using diverse mechanisms. Maintaining metal homeostasis is particularly important when bacteria are exposed to ROS as redox active metals, for example copper and iron, exacerbate ROS toxicity. In this work we have identified and functionally characterised a C. jejuni metal exporter from the cation diffusion facilitator family. Inactivation of the corresponding gene resulted in a metal sensitive phenotype as well as accumulation of elevated intracellular metal levels confirming exporter activity. The mutant strain was also sensitive to ROS and both reactive chlorine and nitrogen species demonstrating a link between metal homeostasis and sensitivity to these components of innate immune defences. The regulation of this exporter gene was investigated through transcriptional fusion of tits promoter to the reporter gene lacZ and incorporation of this construct onto the C. jejuni chromosome. In this reporter strain, b-galactosidase activity was induced by metals consistent with demonstrated metal exporter activity. Furthermore, on inactivation of the ferric uptake regulator gene fur, promoter activity was derepressed in an iron independent manner suggesting an atypical apo-Fur mediated repression of the promoter alleviated by conversion to holo-Fur on iron binding.

B262

Ascophyllum Nodosum seaweed extracts inhibit Influenza infection

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Abstract

Influenza viruses are segmented single stranded negative sense RNA (-ssRNA) viruses in the *Orthomyxoviridae* family and cause respiratory infections. Influenza A viruses (IAV) are responsible for the majority of human disease and 5 pandemics since 1889, the most recent of which was 2009 and the most lethal in 1918 with over 50 million recorded deaths worldwide. Vaccines and antiviral drugs are available however these are often ineffective due to rapid virus evolution. This study focuses on an Enriched seaweed extract (ESE) isolated from *Ascophyllum Nodosum.* ESE was screened for antiviral activity by plaque reduction assays against IAV H1N1 and H3N2 subtypes. Time of addition assays and immunofluorescent imaging were used to help determine the mode of action. The therapeutic potential of the ESE was then explored using differentiated human bronchiole epithelial cells grown at the air liquid interphase and in a murine model challenged with IAV. The data indicates ESE primarily interacts directly with virions, preventing virus cell binding and entry. Interestingly, ESE also inhibits early and late stage of the influenza lifecycle when treatment occurs after cell binding. This inhibitory effect appears to prevent trafficking of viral RNPs to the cell nucleus and release of progeny virus. Intranasal administration of ESE in mice prior to sub-lethal infection with IAV or at the time of infection reduced virus mediated weight loss and viral load in lung tissue. ESE may be a promising broad acting antiviral agent in the treatment or prophylactic treatment of viral respiratory infections.

B263

Methylglyoxal has a bacterial species-specific role in the non-peroxide antimicrobial activity of Mānuka honey.

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Abstract

Antimicrobial resistance poses one of the greatest threats to human health, being currently associated with almost 5 million deaths annually. Despite this alarming situation, the antimicrobial drug pipeline continues to diminish due to the limited success of *de novo* antibiotic discovery. Attempts to combat this crisis have triggered an increased interest in natural antimicrobials, including New Zealand Mānuka honey. Honey in general has known antimicrobial activity, largely attributed to the presence of hydrogen peroxide, however, Mānuka honey also possesses unique non-peroxide antimicrobial activity (NPA). The NPA of Mānuka honey has been partly attributed to the unique presence of methylglyoxal (MGO), a dicarbonyl compound derived from the nectar of Mānuka (*Leptospermum scoparium*) flowers. However, there is uncertainty over whether MGO is the sole driver of antimicrobial activity. In response, we investigated the inhibitory and bactericidal effects of a selection of Comvita® Mānuka honeys with varying MGO concentrations against a panel of bacteria, including MDR *Mycobacterium abscessus* subspecies and *ESKAPE* pathogens. Subsequently, we worked to further characterize the antimicrobial role of MGO in isolation and in combination with Mānuka honey and artificial honey. Our results demonstrate that MGO is unlikely to be the only component of Mānuka honey which contributes to its NPA. Additionally, the antimicrobial role of MGO appears species-dependent, and not dependent on Gram status. By providing greater insight into the antimicrobial role of MGO within Mānuka honey, these results can aid future work into the clinical application and optimal composition of Mānuka honey for the treatment of MDR infections.

B264

BET inhibitors: novel approaches to combatting antimicrobial resistance.

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Abstract

Antimicrobial resistance (AMR) remains an ever-increasing global threat and by 2050 it is estimated that AMR will contribute to 10 million deaths annually. One approach to combatting AMR is identifying novel target sites and processes within the bacterial cell. Bromodomains are specific amino acid sequences in proteins that recognise acetylated lysine residues within other proteins. There are several bromodomain-containing proteins which have a range of biological functions, including the Bromodomain and Extra-Terminal (BET) domain family. BET proteins have a crucial role in regulating gene transcription through epigenetic interactions between bromodomains and acetylated histones in eukaryotic cells and have been implicated in cancer biology and inflammation. Though prokaryotic cells lack histones as defined by the eukaryotic paradigm, histone like proteins (HLPs) have been identified within prokaryotic cells that are thought to contribute to the regulation of gene expression from the nucleoid region. BET proteins therefore represent an attractive target for the development of small-molecule inhibitors (SMIs). Preliminary antimicrobial screening showed that BET SMIs demonstrated activity at <1mM against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* and that activity occurred rapidly. Previous studies have demonstrated low cytotoxicity of the SMIs in eukaryotic cell culture modelling. Investigations are now focussed on further evaluating the antimicrobial and anti-virulence properties of the SMIs, coupled with elucidating the biological mechanisms of activity. This study presents a novel insight into a previously uncharacterised bacterial pathway which may present an attractive target for deploying novel therapeutic interventions.

B265

Ibrutinib enhances the bias of T cell responses towards staphylococcal superantigens sustaining inflammation in chronic lymphocytic leukemia

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Abstract

Chronic lymphocytic leukaemia (CLL) is an uncurable condition of unknown etiology, associated with severe infection morbidity, including sepsis, alone killing ~20% of CLL patients. Treatment with Bruton's tyrosine-kinase inhibitors (e.g., ibrutinib) has improved disease management and T-cell function in CLL, while not compensating for severe infections and patient poor immunization outcomes. We hypothesized that superantigens (SAgs) from endemically diffused *Staphylococcus aureus* (SA) profoundly impair immunity in CLL patients, further aggravated by ibrutinib treatment, driving sustained inflammation. Here, we evaluated the reactivity of T cells and CLL-B cells derived from ibrutinib-treated (compared to untreated) CLL patients, upon chronic exposure to SA SAgs, *in vitro*. First, we found that long-term treatment with ibrutinib reduced naive CD8+ T cells ($p=0.0348$), while promoting higher expression of TIM-3 ($p<0.0001$) associated with lower cytokine responses ($p\leq0.0298$), *ex vivo*. Ibrutinib treatment associated with preferential expansion of memory cells with an exhaustion-phenotype (TIM-3 and PD-1), enhanced by chronic SAg-exposure ($p\leq0.035$). However, these maintained inflammatory cytokine secretion, while staphylococcal SAgs failed to induce regulatory T cells from CLL patients (but not healthy donors, $p\leq0.0461$), irrespective of ibrutinib treatment. While residually activated CLL-B cells appeared in CLL patients (including ibrutinib-treated), these significantly acquired CD38, CD40, CD86, while downregulating CD27, upon SAg-exposure ($p\leq0.005$) even in ibrutinib-treated CLL patients. Thus, we suggest that environmental SAg-exposure deteriorates (ibrutinib-treated) CLL patient immunity, by promoting pseudo-exhausted T cells, which induce/sustain tumour cell activation and chronic inflammation. Our study may help better understand CLL etiology and infection occurrence while improving future diagnostic/prognostic applications.

B266

A novel virulence factor, TcaA, mediates re-modelling of the *Staphylococcus aureus* cell wall

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Abstract

Staphylococcus aureus (*S. aureus*) is a leading cause of bacteraemia, a life-threatening infection of the bloodstream. For the last two decades, the mortality rate from *S. aureus* bacteraemia has remained consistently high, highlighting the need for novel therapeutics to improve patient outcome. In previous work, we used a combination of functional genomics and *in vivo* competition assays to identify the TcaA protein as a novel virulence factor critical for the ability of *S. aureus* to cause bacteraemia. Here, we show that expression of TcaA is induced upon exposure to human serum and that this mediates an increase in wall teichoic acid (WTA) content in the cell wall. We found that this response alters the sensitivity of *S. aureus* to several antimicrobial agents, including peptides and fatty acids present in human serum and several antibiotics with distinct mechanisms of action. To understand how TcaA contributes to WTA biosynthesis upon entry to the bloodstream, we performed co-immunoprecipitation (Co-IP) experiments on cultures uninduced and cultures where cell wall stress is induced by teicoplanin. Collectively, our data suggests that TcaA is a novel protein involved in wall teichoic acid biosynthesis and that the alteration of cell wall architecture by TcaA is critical for the full virulence of S. aureus during bacteraemia.

B267

Immunomodulatory potential of probiotic *Limosilactobacillus* species against *Staphylococcus aureus*.

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Abstract

Staphylococcus aureus (*S. aureus*) is a major pathogen that accounts for approximately 20% mortality rates in the intensive care unit. To improve clinical outcomes, novel therapeutic strategies are needed to prevent its infection spread. In contrast, intake of probiotic *Limosilactobacillus* species mediates beneficial effects, including the synthesis of vitamins, enzymes, and anti-inflammatory compounds. Recent evidence has shown that probiotic bacteria can limit pro-inflammatory responses and inhibit the colonization of *S. aureus* in host tissues. However, the precise mechanisms behind these effects remain unclear. We hypothesise that probiotic rather than pathogenic bacteria-derived products favour immunosuppression that would help limit inflammatory responses. To test our hypothesis, we cultured peritoneal macrophages (pMΦ) and splenic T cells with whole dead bacteria (WDB) and biofilms derived from probiotic *Limosilactobacillus casei*, strain Shirota (LcS), compared to pathogenic *S. aureus*, ATTC43300. The results revealed that LcS WDB did not induce pMΦhyperactivation, unlike *S. aureus* WDB which significantly upregulated costimulatory CD80/CD86 signals ($p \leq 0.05$). In contrast, *S. aureus* biofilm reduced the expression of CD86 ($p < 0.05$) while promoting the upregulation of immunoregulatory PD-L1 ($p < 0.005$), suggesting that staphylococcal biofilms may promote immunosuppression. Concurrently, reduction of memory T cells was observed in biofilm cultures suggesting additional adaptive immunosuppressive mechanisms. Finally, in coculture experiments, LcS products could abrogate pMΦ hyperactivation induced by *S. aureus*, through CD80 modulation ($p < 0.005$). In conclusion, probiotic LcS products can directly dampen the pro-inflammatory response induced by *S. aureus*, relevant for future therapeutic strategies.

B268

Identification of Proteins Degraded by Lysosome-Dependent Processes during Dengue Virus Infection by High-Throughput Proteomics

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Abstract

Dengue virus (DENV) causes the most important arthropod-borne viral disease of humans. Autophagy is a lysosome-dependent process responsible for degradation of cellular components. DENV induces autophagy during infection to enhance replication and evade the innate immune response, however little is known of the host proteins degraded during infection. The study aims to use high-throughput proteomics to identify host cell proteins degraded by the lysosome during DENV infection.

Human Huh-7 liver cells were infected with DENV-2 or mock infected. At 12 hours post-infection the cells were treated with either the lysosomal inhibitor bafilomycin or DMSO (vehicle control) and incubated further for 18 hours. The cells were harvested and protein amounts analysed by Tandem-Mass-Tagging in combination with mass spectrometry.

Bioinformatic analysis of the proteomic datasets identified 502 proteins significantly decreased ≥1.5-fold in the proteomes of DENV-infected cells compared to mock-infected cells. By contrast, only 370 proteins were significantly decreased by ≥1.5-fold when DENV infected cells were treated with bafilomycin compared to mock infected cells. Focused gene enrichment analysis of the proteins stabilised by bafilomycin treatment revealed an enrichment of proteins associated with gene ontology biological process terms including “Autophagy of the mitochondrion”, “Autophagy” and “Positive regulation of cellular component organization”. Proteins representative of these processes including MAP1LC3B, SQSTM1, CALCOCO2, BNIP3, GABARAPL2, SDC4, and GPC3 were selected for Western blot validation. Only the validation of MAP1LC3B, CALCOCO2, BNIP3, SQSTM1, and GPC3 was successful, but provides evidence that proteins involved in innate immune evasion and apoptosis are targeted for lysosomal degradation during DENV infection.

B270

Preliminary investigations of Phytochemistry and antimicrobial actions of extract of *Anogeissus leiocarpus* leaves on some selected clinical Isolates

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Abstract

Aim: *Anogeissus leiocarpus* plant is used for various purpose in some parts of African countries. In addition, antimicrobial actions in the treatment of infectious disease have been reported for extracts of different parts of the plant. The use of leaf extracts in the traditional management of diabetic foot ulcers has been reported. However, mechanisms underlying majority of these beneficial effects are poorly understood. This study investigates antimicrobial activities of *A. leiocarpus* extracts.

Methods: Aqueous and methanolic extracts of *A. leiocarpus* were prepared using Soxhlet extraction (1,2), and the extracts were screened for the presence of key phytochemicals (2,3,4). Antimicrobial activity of the extracts was observed using well diffusion tests and broth microdilution. The diameter of the cork borer was 4mm, standardized inoculum for each test organisms was 10^8 (CFU/ml). The Minimum inhibition concentration ranges from 24ug/ml 0.04ug/ml. Tests were performed in triplicate with incubation at 37^0c for 24 h.

Result:The phytochemical result revealed the preponderance of tannin, phlobatannin, alkaloids, cardiac glycosides, terpenoids, Steroid, Anthraquinone and Saponins. Antimicrobial activity showed aqueous extracts as the most effective. Inhibition for *E. coli was* 18mm, *P.aeruginosa* 18mm, *A.baumanii* 22mm and MRSA 22mm, with concentration of 2.4mg/ml. All the test organisms showed Minimum inhibitory concentration (MIC) at 12ug/ml except for *Acinetobacter baumanii* which shows an MIC breakpoint at 0.09ug/ml.

Conclusion: These preliminary results partly confirm the antimicrobial activity of the leaf extract of *A.leiocarpus* against the four bacterial strains and suggests that further investigations are needed to investigate more antimicrobial activity promise of the plant.

B271

Interactive visual analysis of Campylobacter

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Abstract

Campylobacter is the most common cause of bacterial gastroenteritis amid the human populations. Campylobacteriosis is thus considered to be a major public health concern. *C. jejuni* accounts for about 90% of campylobacteriosis while *C. coli* is responsible for most of the remaining cases.

PubMLST.org is a widely-used open-access genomics reference database, containing an integrated collection of databases comprising phenotype and provenance data (metadata) linked to nucleotide sequence data up to and including genome assemblies, for molecular characterization of many bacterial species. Such epidemiological information is crucial for monitoring, tracking, and responding to cases of *Campylobacter*.

However, genomic data in its raw form is difficult to understand and analyse. The application of visualization and visual analytics together with human factor methods provides an effective means for exploiting big and complex dataset such as genomics data. These techniques can transform such inherently non-visual data into intuitive visual forms that enable users readily to gain insight into, and understanding of, information contained within the data.

Here we present our interactive dashboards to support the exploitation of the *Campylobacter* isolate data hosted in PubMLST. We demonstrate the effectiveness of visual analytics techniques in enabling users to assimilate and understand the high dimensional multi-variate genomics datasets: detecting temporal and geospatial trends, variations and anomalies, the evolution of *Campylobacter*, and sources of infection.

B273

A Closer Look at Multidrug-Resistant *Staphylococcus* spp. in Veterinary Dermatology: Assessing Resistant Bacteria through Indirect Evaluation in Hospital Environments

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Abstract

Veterinary hospitals lack studies on environmental contamination, particularly by *Staphylococcus* spp, a leading cause of pyodermatitis and clinically important for human health. This research aimed to identify *Staphylococcus* spp. diversity in environmental samples from the Dermatology sector of a Brazilian veterinary hospital, evaluating antimicrobial resistance and resistance-associated gene prevalence. Swab samples were collected from various surfaces after cleaning and at the end of the workday. *Staphylococcus* spp. were cultivated and isolated on Mannitol Salt Agar (35ºC – 37ºC for 24 hours) and identified using MALDI-TOF. Antimicrobial susceptibility was assessed by disk diffusion, covering drugs such as penicillin G, gentamicin, tetracycline, ciprofloxacin, sulfamethoxazole, erythromycin, cefoxitin, chloramphenicol, and oxacillin. The broth microdilution test determined minimum inhibitory concentrations (MIC) for penicillin. Eighty-five *Staphylococcus* spp. strains were isolated, mainly *S. schleiferi* (22.3%), *S. pseudintermedius* (16.5%), *M. sciuri* (14.1%), and *S. conhii* (10.6%). Antimicrobial resistance was notable, with 70.6% exhibiting resistance to penicillins, followed by macrolides (27%), aminoglycosides (24.7%), quinolones (14.1%), tetracyclines (12.9%), phenicols (10.6%), and cephalosporins (21.2%). About 32.9% were multidrug-resistant, particularly *S. aureus* (100%), *S. pseudintermedius* (39.3%), *S. schleiferi* (14.3%), and *S. haemolyticus* (14.3%). Penicillin MIC ranged from ≤ 0.015 µg/ml to ≥ 8 µg/ml, with higher resistance post-workday. Resistance to tetracyclines, phenicols, and quinolones was lower after cleaning than post-workday, but MDR prevalence remained consistent. *mecA* and *blaZ* genes were detected in 15.5% and 20% of isolates, respectively. The findings highlight the significance of monitoring antimicrobial resistance in Veterinary Dermatology through indirect analysis of hospital environments with a One Health approach.

B274

“Exploring the efficiency of gut microbiome derived antimicrobial peptides as a potent drug candidate against *Mycobacteria*”

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Abstract

Alternative therapeutic strategies for the treatment of tuberculosis (TB) are urgently required due to the toxicity of available treatments and the increase in antimicrobial resistance (AMR). We investigated the anti-TB activity of three gut microbiome derived antimicrobial peptides (AMPs) Lynronne 3 (Lyn3), CHK-9, 15Sec-D alone and in combination with current anti-tuberculosis treatments using *Mycobacterium smegmatis (M smegmatis)* and *Mycobacterium tuberculosis* H37Rv *bleupan* as a proxy. Lyn3, CHK-9 and 15Sec-D showed a similar activity of 8µg/mL against *M smegmatis* whereas the peptides exhibited an activity of 0.5, 2 and 16µg/mL against the strain *bleupan*. Here, we report a novel combination between Bedaquiline (BDQ) and 15Sec-D with a synergic (FICI = 0.5) treatment effect against *M smegmatis*, where the individual MICs of BDQ and 15Sec-D were reduced by 64-fold and 2-fold respectively (512/16 to 2/8 µg/mL). The BDQ/15Sec-D found to have a rapid killing at 3h. Additionally, the combinations of Lyn3/15Sec-D and Rifampicin/CHK-9 were observed to have an additive effect (FICI = 1) with complete kill and reduction (3-4 log CFU/mL) in time kill kinetics analysis. Lyn3 and 15Sec-D exhibited a fast membrane permeabilization activity (100%, >80%) against *M smegmatis* compared to CHK-9 (<10%). However, combinations showed lower permabilisation suggesting a multimodal action of AMPs when in combination. Overall, the individual and combined antimicrobial effects of Lyn3, 15Sec-D, CHK-9 with BDQ and Rifampicin on *M smegmatis* indicate that these gut microbiome derived AMPs are promising therapeutic candidates for development for TB treatment in the post antibiotic era.

B275

Do models of fungal infections caused by *Candida auris* reflect what happens in patients?

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Abstract

Candida auris is a recently emerged fungal pathogen, causing life-threatening fungal infections in seriously ill patients. Similar to other fungi, systemic infection is often modelled in mouse models, but are they are accurate reflection of what happens in the human host? Systematic reviews will be used to determine the risk factors for these infections and if there are differences seen for fungal strains from different clades. How infections are modelled in animals will also be reviewed and their relevance to risk factors in the host will be explored.

B276

Detection of Burkholderia pseudomallei with CRISPR/Cas12a using the specific marker orf11 of the first type III secretion system (T3SS-1)

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Abstract

Burkholderia pseudomallei is the etiologic pathogen of a severe infectious disease known as melioidosis. The disease in humans ranges from asymptomatic to focal infection and could be life-threatening by rapid fatal septicemia. An early diagnosis of the disease could decrease the fatal rate of the patients. Currently, CRISPR/Cas12a technology is an attractive tool for infectious disease diagnostic applications. The detection depends on the nonspecific endonuclease activity of Cas12a which is binding to a specific target DNA via programmable guide RNA. In this study, we developed a rapid detection of *B. pseudomallei* using CRISPR/Cas12a. The open reading frames: *orf11* from T3SS-1 gene cluster of *B. pseudomallei* , the specific marker distinguishing from another closely related species *Burkholderia thailandensis*, was selected for gRNA design. The DNA of *B. pseudomallei* was extracted from the colony suspension by boiling method. The results revealed that the digestion reaction involving the gRNA specific for *orf11* generated DNA fragments. This finding suggested that the newly designed gRNA was specific to the target DNA sequence leading to the digestion activity by Cas12a enzyme. Additionally, the target-activated CRISPR/Cas12a cleavage activity was verified based on signal amplification of ssDNA-FQ reporter. The result revealed that an increased generation of fluorescence signal, which was observed in the wild type *B. pseudomallei* strain K96243 and *B. pseudomallei* isolated from clinical sample but not in *B. thailandensis*. This concludes that the newly designed gRNAs of *orf11* could specifically detect *B. pseudomallei*, but not other pathogens and can be used for the development of a rapid diagnostic tool for melioidosis.

Keywords: *Burkholderia pseudomallei ,* CRISPR/CAS12a, melioidosis

B277

Benchmarking Large Language Models on Antibiotic Resistance Knowledge

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Abstract

Background: Large Language Models (LLMs) such as ChatGPT have revolutionized the field of natural language processing with their broad capabilities. However, their proficiency within specialized fields, particularly in the biomedical sector, remains unproven. With the stakes involving human lives, the precision of these models must be evaluated rigorously.

Objective: This study aims to benchmark LLMs, focusing specifically on their knowledge and handling of antibiotic resistance (AMR), a critical area where accuracy can be a matter of life and death.

Methods: We developed a comprehensive dataset by manually curating AMR-related question-answer pairs from authoritative biomedical sources, including PubMedQA and MedMCQA, as well as USMLE and other public databases. We compared the responses from ChatGPT against those from specialized models trained on biomedical literature, namely BioGPT and PMC-LLaMA.

Results: The comparison revealed distinct performance variations across the dataset. While ChatGPT occasionally provided more detailed responses, it struggled with complex queries, often deferring to clinical experts. In contrast, biomedicine-specific LLMs showed greater consistency in responding to intricate questions. However, instances of dangerously incorrect information from these specialized models were also detected.

Conclusion: Our findings underscore the need for continuous enhancement of LLMs with a strong emphasis on their ability to estimate the reliability of their responses. This is vital in applications like AMR, where the cost of misinformation can be severe. Ensuring LLMs' reliability in such niches is paramount before they can be deemed fit to assist healthcare professionals.

BLOCK B

Session : Education and Outreach Symposium

B278

Laboratory Skills Portfolio – A Voluntary Fundamental Laboratory Skills Development Program, University of Lincoln, UK.

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Abstract

An extra-curricular laboratory skills development program, “Laboratory Skills Portfolio (LSP)”, has been designed and implemented in response to a perceived lack of student confidence and competence when learning in laboratories. The LSP is a series of voluntary, interactive, laboratory activities that run in parallel with undergraduate and postgraduate laboratory teaching, that focus’ on fundamental laboratory skill development, in a psychologically safe environment, for each individual student. Students are encouraged to practice skills, identified through self-assessment, from a range of topics such as: microscopy, microbiology and solution preparation. Sessions run on Wednesday afternoons and are open to all students, at all levels and on all programs, in the School of Life and Environmental Sciences.

Ethical approval was sort and granted (UoL-14313) to ask students two key surveys which explore student confidence with fundamental laboratory techniques both before and after engaging with the LSP. 58/75 students have completed the survey before attending the event and feedback has been positive. Students complete an activity book that is highly detailed and that has a, “Have I done this right?” section, to test understanding after each activity. Upon successful completion of the activities, these are signed by a member of staff, to produce a bespoke certificate per student. The certificate demonstrates attendance on an extra-curricular course that students can include on their CV’s when searching for future employment. Students will be asked to complete a survey after engaging with the LSP and the changes in student confidence recorded, feeding directly into future LSP development.

B279

Using generative AI in Microbiology teaching encourages students to self-evaluate

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Abstract

Generative AI is a rapidly emerging technology which can be implemented as a teaching tool in the Higher Education (HE) environment. This research sought to exploit how Microbiology students perceive AI to encourage self-evaluation. During a teaching intervention, students were asked to evaluate essays, some of which were generated by ChatGPT 3.5. The metric edits per minute was used to measure the evaluative behaviour of students. Microbiology students were 53% more critical of essays which they knew they were AI-, rather than student-, generated. The intervention resulted in students being 2x more critical of their own work, and those who completed the intervention attained 5% higher in the following assessment. The project presents an effective method for engaging Microbiology students with generative AI. It also encouraged critical student-led discussion around the role of AI in HE, feeding into the wider conversation on the opportunities and challenges presented by generative AI to Microbiology HE teaching and learning.

B280

Engaging the public on Antimicrobial Resistance (AMR); a 'classical' approach

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Abstract

Antimicrobial Resistance (AMR) is a complex and contested area in both public health and public engagement with science. Despite the pressing urgency of raising awareness of AMR there is a paucity of accessible materials that address the history, complexity, depth and range of AMR research and AMR implications.

Drawing on history of science, science and technology studies (STS) ethnographies of microbiology laboratories, interviews with public health scientists, and classical Greek theatre this presentation reports on the construction of a play that recounts the story of *Escherichia coli*'s role in the foundation of microbiological research and its ongoing role in public health interventions and processes, and AMR research.

The play, funded by Wellcome, is designed to engage a wide range of audiences – specialists, general public, school students, public health officials – and is designed to be 'read at different levels' such that audiences can interact with different elements to explore and reveal the complexities of microbiological research pertaining to AMR.

B281

Proposal to integrate higher-order thinking skills and democratic pedagogy into online proctored exams for STEM learners in higher education institutions using the OPERHOT platform.

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Abstract

An appropriate exam system for STEM students that test higher-order thinking skills such as applying, creating, evaluating, and analyzing in alignment with Bloom's Taxonomy was proposed. Empathy interviews with educators suggest that in Higher educational institutions (HEIs), all three strands of democratic STEM pedagogy are essential for science teaching and learning including student voice, shared and transformational authority, and STEM criticality. For HEI, these strands should be also reflected in the assessments of students. It has been observed during the pandemic, that without an appropriate proctored online system of examination in place, accurate or fair assessment of students can be challenging. This is in sharp contrast to the offline mode of examination, which can be monitored. During the COVID-19 pandemic, many students do poorly in higher-order thinking questions and answers to higher-order thinking questions are often identical among students in the unproctored online examinations or insufficient proctored system of examinations in resource-constrained settings. As a result, there is an urgent need to revamp the online system of proctored examination in alignment with STEM criticality and student voice. Here we propose the development of a proctored online exam system integrated with random higher-order thinking questions (OPERHOT) for every student. OPERHOT randomly assign higher-order thinking questions to students in a timed manner and require students to keep their camera on during the time of assessment. Implementing this system will facilitate proper assessment of students in the online mode. Furthermore, this system will strengthen the online system of education and assessment in HEIs.

B282

Unravelling Biofilms: Crafting Solutions for Infection Prevention

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Abstract

Biofilms are a significant challenge in the context of Antimicrobial Resistance (AMR). However, public understanding of biofilms is very limited. As part of a community outreach project, through the medium of knitting, crochet, and stitching, we brought biofilms to life with the help of community craft groups across Sheffield.

The project communicated a research project which developed an antimicrobial impregnated catheter coating to prevent uropathogenic *Escherichia coli* infection. At each session, community members created bacteria out of yarn and attached them to the growing biofilms. Alongside this, the session served as platforms to share insights into biofilms in daily life, the use of antibiotics and the future of antimicrobial resistance.

Taking discussion of biofilms out into the public and linking it to an artistic activity meant that we reached a different group who would not necessarily attend specific science outreach events. The impact of the project on people's knowledge within the area of biofilms and AMR was explored through questionnaires and through thematic analysis of the unstructured discussions which occurred during the event. These will be used to develop the focus of future events.

This project is funded by the Microbiology Society Champions Scheme.

B283

Building Alliances: An Interdisciplinary Approach to Learning About the Microscopic World

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Abstract

Interdisciplinary learning encourages students to explore a selected topic from different specialist areas. Science and Art are two such disciplines that are often considered to be opposing yet can form a powerful and complementary pair when used effectively to work on a common theme. In recent years, this has been demonstrated through projects focused on the use of tools for the visualisation of molecular level scientific processes as well as the creation of artistic models of microorganisms. Interdisciplinary projects have also been used to uncover the ethical implications of acquiring accessible genetic information from everyday objects, bringing to light the increasing desire to wholly explore topics using interdisciplinary media and knowledge.

This collaborative work involves investigators and cohorts at the School of Science and Technology, Nottingham Trent University, and the Games Art Department at Confetti Institute of Creative Technologies, Nottingham. By working together over the course of a three-month period, students have researched a range of cell types as well as their organelles and functions. Expertise in comic book art and drawing has been used to introduce creative elements of storytelling and concept art design, allowing for the visualisation of cells and their organelles in a memorable way. Science students have gained experience in creative ways of explaining scientific content and vice versa. Most importantly, this work highlights the challenges and benefits of interdisciplinary collaboration. In this way, this study also introduces real-world elements of working collaboratively as methods for developing communication and professional identity in students.

B284

Using Photovoice to engage students in a non-major microbiology course

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Abstract

It is becoming increasingly difficult to engage and encourage critical thinking and deeper learning in students who participate in non-major subjects. At Dundalk Institute of Technology, Microbiology is taught as a non-major subject in many different programmes ranging from agriculture, veterinary nursing, bioscience and biopharmaceutical science. In an attempt to inspire and increase student engagement in microbiology, this study used Photovoice as a pedagogical tool. Photovoice is a participatory action research methodology used in community-based research in a variety of different areas, where participants are invited to use photography to demonstrate their point of view. Third year bioscience students were invited to take two photographs which represented "microbiology in our world" and to write a narrative paragraph explaining why the photos were taken. Participating students were invited to participate in a focus group to ascertain the effectiveness of Photovoice as an engagement tool. Overall, students reported a positive experience of using Photovoice; analysis of the focus group data resulted in themes of choice, creativity, critical thinking and research skills emerging. These findings suggest that Photovoice is an effective way of engaging students in microbiology as a non-major subject. Examples of some of the images as well as some extracts of the narratives will be presented.

B285

How much do we know about gut microbiota? An international questionnaire.

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Abstract

The exploration of the gut microbiota has rapidly advanced as a scientific discipline in the last two decades. This substantial growth has revealed the extensive impacts of the gut microbiota on aspects such as host nutrition, health, and behavior, transcending various fields including biology, medicine, and ecology. The intricate interplay between daily choices, life events, and the composition of the gut microbiota makes it an intriguing topic for scientific communication. Scientific communication involves tailoring the conveyance of scientific knowledge to diverse audiences. Nevertheless, this communication has expanded beyond academic circles, reaching diverse channels where misinformation can emerge.

We conducted an international survey to evaluate both the public's and healthcare professionals' understanding of the gut microbiota and the accuracy of the information available. 1288 participants from 54 countries completed the survey in one of the 11 languages available. More than half of the participants (53.5%) worked in healthcare or research professions while 46.5% of the responses came from non-specialised public. Most of the participants (88%) have heard about the concept of "gut microbiota" before, mainly in the media (43.57%) and social media (43.89%). However, only 22.98% could associate healthcare alterations related to some extend with gut microbiota correctly. Similarly, participants were not clear on what information can be obtained from a gut microbiota analysis or how to order it.

Ultimately, these insights will guide the development of more effective strategies for communicating gut microbiota research to both the general public and healthcare professionals.

B286

Science communication skills utilised in expert witness work – can outreach skills help settle legal disputes?

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Abstract

Science education and outreach is a rewarding aspect in many science-based career routes, especially for those working in further and higher education. While the majority of science education and outreach is aimed at and delivered for school children or the general public, far less is delivered for other professional sectors especially in formats that align with outputs from those sectors. Science education and effective communication is important to a number of professional sectors including the civil service, the food & beverage industry and both the criminal and commercial legal sectors, to name a few examples.

The role of expert witnesses in law is a long established approach and a key example of two professions working together under one jurisdiction. The COVID-19 pandemic resulted in what is likely to be a large number of civil and commercial legal disputes requiring microbiologist or medical expert witnesses to analyse and interpret technical evidence. While expert witness short courses are widely available, what are the key attributes required to act as an expert witness under legal instruction? Effective verbal and written communication are essential, but how is the approach different to communicating in lay language?

Here I outline the role of an expert witness in an arbitration setting, drawing from my own experiences in cases where COVID-19 has resulted in significant losses of income in the maritime industry. I summarise some versatile science communication and education approaches utilised in this interesting setting, as well as opportunities for microbiologists looking to augment their professional development.

B287

Needs assessment for pathogen genomics training for health and research professionals in the UK and Ireland

Alice Matimba ORCID iD[1], Monica Abrudan ORCID iD[2,1], Dusanka Nikolic[1], Diana Connor[2], Katherine Kaldeli[1], Rachel Berkson[1], David M Aanensen[2]

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Abstract

The COVID-19 pandemic underscored the importance of integrating genomics with epidemiological and clinical data in routine or emergency surveillance of infectious disease.

Concomitantly, the pandemic also highlighted the global paucity of trained professionals and the need for targeted and formal approaches to developing a workforce that can shape and deliver the value of genomics for additional pathogen paradigms, and the appropriate public health responses, informed by the additional precision afforded by genomics.

To assess the training requirements in data science and pathogen genomics for healthcare and research professionals in the UK, we implemented a comprehensive needs assessment. This involved a mixed-methods approach, encompassing interviews with experts, surveys, discussion forums, and workshops. We engaged ten experts in the field, including infection control specialists, public health consultants, and hospital managers, to gain a broad perspective on the current educational needs.

Applying thematic analysis, we identified key training needs, important stakeholders, challenges in learning pathogen genomics, preferred training formats, and how pathogen genomics knowledge is applied in various healthcare and research settings.

Our findings informed the design and development of tailored train-the-trainer courses, contributing to the successful training of more than 70 professionals from diverse backgrounds, the establishment of a pathogen genomics community of practice and initiated a pathogen genomics competency framework.

Acknowledging the limitations due to sample size and potential biases in responses, this needs assessment paves the way for more nuanced and effective training methodologies in pathogen genomics, informing future program designs and improvements in the field.

B288

Microbiology labs increase students' perceptions of community and confidence to verbally contribute to class discussions.

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Abstract

The presence of a strong community within educational settings significantly benefits students by fostering belonging, retention, and motivation. It promotes inclusivity, positively influences mental well-being, and holds particular importance for at-risk learners. Relationships among community support, motivation, and engagement are complex and multifaceted.

A focus group formed of 2nd year Microbiology students were asked to reflect on their 1st year experiences generally, and specifically about examples of events or activities which positively or negatively affected their sense of cohort community. Laboratory classes were highlighted by the students as significantly increasing their sense of community while large, multi-program lectures negatively influenced their sense of community. They felt that working in a laboratory setting on a shared problem as part of a pair or small group enables them to "*really get to know people*" and increase familiarity.

I also investigated the students' perceptions of the intersection of verbal communication confidence and cohort community. Contrary to common belief, verbal communication reluctance is rarely just a language confidence issue. It is also influenced by the fear of judgment from one's community. The students stated an increase in confidence of verbal contribution to class discussion within their community, but also that this is a complex situation.

The findings from this focus group have wide ranging applications, but emphasises the importance of research-based learning in higher education.

B289

Enhancing student's mathematical skills with smart worksheets

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Abstract

Higher education teachers are continually exploring innovative approaches to facilitate active learning within our frequently expanding cohorts. A key area which students are known to find challenging is appropriate use of calculations and data analysis. Our study involves the implementation of an interactive online assessment tool within a second year undergraduate 'Microbiology and Immunology' course. We have been working with digital learning company, Learning Science, to develop 2 independent 'smart worksheets' as part of a blended learning approach to formative and summative assessment of key calculation and data analysis methods. These interactive worksheets are embedded within our virtual learning environment (VLE), Moodle, and present students with a unique dataset for analysis, as well as a series of questions for them to work through independently in their own time. Each worksheet is automatically graded within the VLE and students are given immediate feedback on their responses as they work through each question. In this way, feedback is given at every stage of the calculation to catch any errors quickly, as well as providing advice on any errors and how to correct them. Students are therefore able to achieve a deeper level of understanding of key calculations they are required to learn as part of this course. Furthermore, autograding has benefits for staff by reducing marking and feedback time. We expect that the long-term impacts from this study will encourage the implementation of similar innovative teaching modalities in other biology courses.

B290

Using LinkedIn to Get In: Evaluating the impact of LinkedIn groups on undergraduate career awareness and confidence in networking.

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Abstract

The extent to which individuals interact online has expanded in recent years, with social networking a major aspect of most people's lives. Development of a professional online presence has become an important aspect in most sectors, and potentially a challenge for students preparing to enter the workplace. LinkedIn is a globally recognised professional network, enabling individuals to interact within a professional environment. However, it remains uncertain whether students are aware of its benefits.

The University of Glasgow initiated a closed Microbiology LinkedIn group, limited to staff and current/former Microbiology students, aimed at promoting career diversity and enabling students to network and form valuable connections in their chosen field. However, levels of student awareness and engagement with this platform, and student confidence in professional networking remains unclear and could influence students' abilities to gain real-world skills. This project evaluated how confident third year undergraduate students were in using LinkedIn as a professional networking platform and whether it improved their awareness of career opportunities.

Students joined the closed group and conducted an interview with an alumnus of their degree, allowing exploration of career prospects before presenting their findings to their peers. Confidence in using LinkedIn for networking increased following this session, with all students agreeing the inclusion of such a workshop in the curriculum is useful for exploring career options as they left with 15-20 different pathways open to them. LinkedIn has potential for being effective in supporting transferable skills such as networking, regardless of social background and mobility.

B291

Assessing the Impact of the Bioskills-at-Home Kit Teaching Innovation at Nottingham Trent University

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Abstract

Practical laboratory training is a key aspect of students' learning in Biosciences. Particularly fundamental for first-year students, it aids the development of critical skills relevant for the rest of their university years. The COVID-19 pandemic and restrictions however made this learning objective difficult to achieve. The 'Bioskills-at-home' kit was created to support first-year students in developing core skills and building online learning communities. The kits contained equipment and activities in microscopy, pipetting, data handling and experimental design. While it was clear that the bioskills kit was beneficial to students, a significant challenge was the lack of engagement which led to embedding its delivery within the tutorial system, post-pandemic. Therefore, we sought to explore students' experiences from two cohorts - pandemic (2020/21) and post-pandemic (2022/23)- including ease of use, enjoyability, motivation, perceived benefits, support and barriers to engagement. Data was collected using survey and focus group and the results show that the kit supported students' learning, especially with the compulsory practical techniques test. They had an enjoyable experience, found it supportive in the development of technical skills and understood how these skills linked to their taught material. Some challenges included being unclear where to find additional information, lack of access to smartphone/internet and insufficient time. Students from 2020/21 cohort felt there would have been better engagement with the kit if some structured timetabling sessions facilitated real-time collaborative activities via Teams. Moreover, the in-person delivery through tutorials seemed to have improved students' engagement with their kits and their confidence in using them.

B292

Tackling TB: Dundee scientists fighting the killer cough

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Abstract

What links the University of Dundee's Drug Discovery Unit and Dundee's industrial heritage?

This was the question we asked ourselves as we began working on an exhibition with a team from the University and Verdant Works, Dundee's jute mill museum. From a series of conversations with scientists and heritage experts, one theme quickly jumped out - Tuberculosis (TB).

There are a fascinating series of parallels between the 2 places, only a few hundred metres apart. Dundee used to be one of the leading places in the world for jute production - and also used to have appalling slums where people lived and died with TB. Now both jute production and TB are primarily found elsewhere in the world, and Dundee has a new industry - biotechnology. Nonetheless, many of the same social issues still exist, and the need to empower people with knowledge of science is as urgent as ever.

In this panel event, we plan to tell the story of our exhibition and the people behind it. We will feature Dr. Laura Cleghorn, our lead TB scientist from the Drug Discovery Unit, who will give an update on the exciting research developments in the past few years. We will also hear from both our lead curator, Sophie Hinde, and our museum consultant, Lynsdey Clark. Chaired by Ali Floyd, Public Engagement Manager for the Wellcome Centre for Anti-Infectives Research, which the Drug Discovery Unit it part of, we hope delegate will find the session insightful and inspiring in their own engagement journeys.

B293

Enhancing Digital Accessibility in Biosciences Laboratory Sessions: A Student-Centric Approach

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Abstract

In an era of rapid technological advancement, this study addresses the intersection of biosciences education and digital accessibility. Focused on enhancing the quality of laboratory sessions, our project explores the integration of assistive technologies and visual elements to facilitate inclusive learning environments. A survey revealed a significant gap in students' awareness of assistive technologies. Our initiative extends beyond identifying the issue; we've developed 'top tips' for teachers conducting lab introductions, promoting a more inclusive learning environment. These outcomes not only contribute to biosciences teaching practices but also offer valuable insights for educators seeking to harness technology for inclusive pedagogy.

B294

An Integrated Training Approach to Accelerate Capacity Building in Pathogen Genomics

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Abstract

Background and objective

Infectious disease outbreaks and antimicrobial resistance demand full exploitation of genomics technologies to support clinical management and pathogen surveillance. Therefore, there is a critical need for genomics expertise to enable effective application in healthcare. Towards scaling up capacity in pathogen genomics data science in clinical microbiology, we developed a Train-the-Trainer (TtT) course which integrated pedagogical and genomics concepts (T3).

Methods

Targeted at research, healthcare and training professionals, the course aimed to build upon their expertise in pathogen genomics and provided a framework for designing and building training resources. The integrated T3 curriculum involved:

1. Developing a guideline on how to train experts to facilitate training others using active learning strategies and applying pedagogical concepts to genomics and data science learning activities.
2. A 4-day course integrating training design concepts with genomics workflows from sample to genomic data interpretation for outbreak detection and surveillance.
3. A project-based learning approach where trainees were mentored in developing their own training materials, training facilitation and community development.

Results

Since October 2022, we have delivered three runs of this course to 69 trainees including clinical, biomedical and public health scientists, epidemiologists and bioinformaticians. To date over 20% of trainees have reported improved educational practice, developed in-house training for colleagues and trained other healthcare professionals.

Conclusion

The integrated TtT strategy is an effective method for empowering research and health experts with tools and resources to cascade their knowledge and skills to their colleagues and multidisciplinary peers, thereby accelerating capacity for genomics in healthcare.

B295

Beyond Lectures: Leveraging Competition, Peer Discussion, and Real-World Scenarios in a Digital Card Game to Enhance the Learning of Microbiology and Immunology Concepts

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Abstract

Teaching the complex interactions between hosts and pathogens is a fundamental yet pedagogically challenging aspect of medical education. The intricate mechanisms of the immune system can pose a significant barrier to students' understanding of infectious disease diagnosis and treatment. To address this, we have designed a digital card-based competitive game called *Micro-Immune Battles*, aimed at more actively engaging students with microbiology and immunology concepts.

The game structures learning around a wide range of infectious disease scenarios, including viruses, bacteria, fungi, and parasites. Student teams are provided with digital "immune system response cards" that represent various immune elements. Teams must digitally construct sequential card cascades that correctly show how the immune system responds to the specified pathogen in the scenario. This reinforces the temporal progression of immune responses, whilst encouraging the application of theoretical knowledge to practical cases. Scoring is determined by the accuracy and speed of card placements, incentivising rapid yet correct synthesis of knowledge. Points are deducted for incorrect placements, introducing an element of calculated risk-taking and critical reasoning.

The game's competitive nature fosters an interactive and engaging classroom environment. At the conclusion of each round, the highest scoring team must verbally justify their card and placement choices to their peers, facilitating both peer-to-peer learning and reflexive understanding. Preliminary student feedback suggests this approach makes host-pathogen interactions more enjoyable and comprehensive to learn.

B296

“Microbiology Scramble” an interactive game to engage audiences with Microbiology

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Abstract

The use of games for effective science education has grown, providing intense engagement and concentration unmatched by other methods. Games support increased interest and motivation, generating positive attitudes toward the subject. The "Microbiology Scramble" tabletop game, a modified version of Scrabble, was developed to make microbiology learning fun, interactive, and accessible to diverse audiences.

The game contained 50-70 large wooden tiles with individual letters, which was tested at the 2019 and 2023 Swansea Science Festival (SSF). Participants were invited to spelling as many microbiology words as they could without a board. Players were also supplied with a ‘key terms glossary’ to help them come up with relevant words, although they were also free to come up with their own words such as bacteria, influenza, HIV etc, fostering an enjoyable and interactive experience. The game, lasting 15-20 minutes, was played individually or in groups racing to form the most microbiological terms before running out of tiles. Feedback from participants, represented as a word cloud, indicated overwhelmingly positive experiences. In 2019, all participants rated the game as 'Excellent' (n=13), and in 2023, most rated it 'Excellent' (n=17), with three scoring it as 'Good' (n=3).

This evidence suggests that games like "Microbiology Scramble" serve as effective tools for teaching microbiology to diverse audiences, promoting engagement, teamwork, and enhanced learning. Future efforts focus on integrating the game into formative assessments for university science students, particularly for challenging or less engaging topics, to further improve learning and academic performance.

Swansea Research Ethics Approval Number 2202378386905.

B297

Keep your WITTs about you to advance Infection and immunity teaching!

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Abstract

There is a significant interest in studying infection and immunity with increasing student numbers on undergraduate and postgraduate taught courses and modules. Infection and immunity research rides high, but who and how will we train the next generation? One solution is through 'simulations' as their use has been steadily rising in popularity in the biosciences, not only due to the COVID-19 pandemic restricting access to physical labs and equipment but also in the face of rising student numbers.

This work describes the development and implementation of a novel, open-access interactive set of simulations, the Welsh Immunology Teaching Toolkit (WITT). This has been used to not only supplement laboratory classes but to enhance the student learning experience. The resources include fully interactive lab methods such as Flow Cytometry, ELISA, SDS-PAGE, cell culture, lab safety, equipment training and revision quizzes. Assessments scores associated with this toolkit resulted in a much broader range of the marking scheme acting as an excellent discriminatory for student ability. Furthermore, the return to face-to-face teaching recognised that these resources provide an opportunity to 'flip' lab teaching, meaning in-person time can be more hands on with less need to review basic concepts during lab sessions.

WITT can play a crucial role in the student learning cycle by providing a rich, engaging learning environment. These simulations gained popularity during the pandemic, but now they need to be used to supplement hands-on laboratory experiences to ensure that students acquire the necessary kinematic skills expected of a successful graduate.

B298

Is language a barrier for engaging a linguistically diverse primary school in Microbiology?

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Abstract

The English language has allowed the advancement of science as individuals around the globe collaborate and communicate findings in a universal language. However, only 15% of the world's population speaks English, with just 5% being native speakers. This could create a barrier between most of the world and the scientific community, however the pursuit of knowledge should not be bounded by language. We need to actively ensure Microbiology is accessible to citizens globally, especially aspiring young people. This is increasingly important as Scotland is becoming more linguistically diverse.

Lessons based on infection biology were delivered to a Primary 3 class in Glasgow, investigating the role language has on enthusiasm and engagement in science. This cohort of pupils were grouped into three categories; New to English, Developing English, and Fluent, with the impact and diversity of microbes taught using visual aids and hands-on experiments to encourage individual exploration. Knowledge and understanding were measured using pre- and post-questionnaires.

Pupils in all groups were quick to participate and confidently present their findings when prompted. Recognition of vocabulary and knowledge of microbes increased uniformly across all English levels. Enthusiasm in all groups consistently increased following the active lessons. Learning Microbiology can occur independently of English language level, indicating the barrier between language and science can be prevented and shouldn't hinder future scientists around the globe. It's important to be aware of potential barriers non-English speakers may face and how we can support them and our surrounding areas in accessing the scientific community.

B299

“Give Me a Name” or how to create synergies between A-level students from humanities and science programmes for the assignment of scientific names to novel bacterial species

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Abstract

Bacterial systematics is the scientific study of the diversity of bacteria and their evolutionary relationships. The classification of bacteria adheres to a hierarchical structure in which names are assigned according to a set of strict rules determined by the International Committee on the Systematics of Prokaryotes (ICSP), notably emphasizing the use of Latin or Greek for nomenclature.

In order to promote interest in scientific knowledge among A-level students and underscore the importance and applicability of classical languages (Latin and Greek) in science, we conducted an educational activity under a contest format titled “Give Me a Name". Thirty-five students from the A-level programmes in Humanities and Sciences worked together on assigning scientific names to four newly discovered bacterial species currently under investigation by IFAPA. Over the course of five sessions, students learned about microbial characterisation and the fundamental nomenclature rules outlined in the International Code of Nomenclature of Prokaryotes by ICSP.

The names proposed by the students underwent evaluation, and the newly named species will be published in The International Journal of Systematic and Evolutionary Microbiology (IJSEM), the official journal of the ICSP. Sponsored by the British Society for Microbiology and endorsed by the Editor of the IJSEM Name Validation List, the activity garnered significant enthusiasm among students and received widespread media coverage. Given its success, future editions of the competition will be extended to other interested institutes and countries.

The contest is funded by RYC2019-028468-I (MINECO, Spain) and counts with the support of the British Society for Microbiology and ICSP.

B300

Five years of Coccus Pocus scary story competitions: what a ride!!! And… what's next?

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Abstract

Coccus Pocus is a nationwide scary story competition about biofilms and antimicrobial resistance that was first launched by the University of Hull in October 2019 and run annually until October 2023. It is part of the #BiofilmAware campaign of National Biofilms Innovation Centre (NBIC), which aims to raise public awareness about the importance of microbial biofilms. During this period, we established a solid network of 15 Coccus Pocus ambassadors in universities and schools who encouraged their students to participate. Entries came from places beyond the UK too, such as Brazil, Denmark, India and Ireland. In 2019, there were only 3 entries, but numbers increased to 4 in 2020, then to 19 in 2021, 19 in 2022 and 10 in 2023 (55 in total). The last three years, we also had submissions from secondary school pupils. First prize winners in the 18+ age group were from the University of Hull (2019), University of Birmingham (2020), University of Glasgow (2021), University of Newcastle (2022) and Asutosh College (affiliated to the University of Calcutta, India; 2023). Young 1st prize winners (aged 12-17) were from Engineering UTC Northern Lincolnshire (2021) and St Peter's Catholic School, Surrey (2022. 2023). Feedback questionnaires were completed by the participants, which showed that they all found the competition very interesting and useful, allowing them to sharpen their creative writing skills and explore key microbiology topics. For the future, we are considering publishing the 24 winning stories as an anthology book or even taking these stories to the theatrical stage!!!

B301

PREPing to learn well: embedding academic literacy for first year biomedical students

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Abstract

In 2022, in line with the wider community, colleagues in Sidney Sussex, a college of the University of Cambridge, noted several issues that impact our student community:

- The need for increasing provision of embedded academic and wellbeing transition support for new students, particularly given the SARS-CoV2 pandemic
- The particular relevance of such provision to our commitment to widening participation.

In 2022 freshers started their degree one week early for a programme of preparation for their academic studies, "Prep Week". This week incorporated activities themed around academic literacy and belonging alongside the NHS pillars of wellbeing. Within this wider context, biomedical students across cognate subjects including Natural Sciences, Medicine, Veterinary Medicine and Psychology and Behavioural Sciences were brought together for sessions on getting the most out of their teaching modes, creativity, problem solving, skills for independent study, consolidation, using feedback effectively and reflective practice. Of those who provided feedback at the end of the week, 99% knew people within and outside of their subject, 83% felt less anxious about starting university, 90% knew where to go if they had an academic issue and the 69% felt more prepared to study their subject and 85 % found the skills sessions useful.

In 2023, feedback was considered and pedagogical underpinning of approaches enhanced. New approaches included the use of a reflective portfolio, vision exercises, tailored problem-solving and codelivery with students from relevant subjects. We will present feedback from PrepWeek 2023, and discuss evaluation, embedding and aligning academic literacy within collegiate Cambridge first year education.

B302

Tiny forests: opportunities for connecting primary school pupils with nature and microbiology

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Abstract

In a nature-depleted world, it has become harder for children to connect with and explore wildlife, let alone understand how microorganisms fit into the picture. To address this, in February 2023, a 'tiny forest' was planted at a primary school near the University of Essex to provide opportunities for pupils to interact with nature. Alongside learning about animals and plants, this tiny forest opens up opportunities to teach children about the microbiology of a woodland ecosystem, from the fungal life cycle to the microbiomes of trees, soil and the air we breathe. Therefore, with input from local teachers and funding from the Suffolk & North East Essex Integrated Care System and local partners, we are developing a 'toolkit' to give educators the resources they need to engage pupils in science-based activities that complement and fit within their National Curriculum learning. This presentation will highlight the microbiology-focused activities included in the 'toolkit' and delivered during several 'Science Fun Days' for primary school pupils hosted at the University of Essex. The impact of the Science Fun Days on pupils' enjoyment of science, confidence and scientific career aspirations will be reported.

B303

Collaboration between Archaeology and Microbiology

Alain Richard

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Abstract

Archaeology and microbiology can be converging disciplines, for instance, using metagenomic tools (Warinner *et al.* 2017). Here are some examples.

The study of past microbiomes can reveal the microbial diversity of ancient gut contents before the industrialization of food production. Research on medieval latrines concluded that DNA preservation can occur after a few centuries, allowing the identification of gut microorganisms (Sabin *et al.,* 2020). Modifications of the human microbiome have been observed in modern diseases such as inflammatory bowel disease, colorectal cancer, type 2 diabetes, and obesity (O'Toole, 2017). It is conceivable to gain insight on healthier modern microbiomes by studying past ones.

Various samples from archaeological remains have demonstrated the evolution of microbial pathogens, helping in a better prediction of their modifications.

Another field of interest is the conservation of archaeological sites from microbial degradation.

A common virus can be a tracker of past human migrations (Pavesi, 2005). The human polyomavirus JC (JCV) is a small DNA virus; the infection is widespread. There is a close relationship of JCV in Native Americans and northeast Asians, substantiating evidence obtained by archaeology.

Current and future opportunities of the cooperation between both disciplines will be discussed.

O'Toole, P.W. (2017) *Microbiology Today,* 44:2, 74-76.

Pavesi, A. (2005) *Journal of General Virology,* 86:5, 1315–1326.

Sabin, S. *et al.* (2020) *Phil. Trans. R. Soc. B,* 375:20190576

Warinner C. *et al.* (2017) *Annu. Rev. Genom. Hum. Genet.,* 18: 321–56.

B304

Modelling Virus Nano-machines for Outreach and Engagement

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Abstract

Representational models are essential tools to help describe, explain, and reason abstract scientific ideas, aiding in the understanding of concepts that cannot always be seen. Over the past five years, our work has focused on developing hands-on mechanical models of viruses that demonstrate virus structure and function for use in educational outreach and engagement. These 3D physical models enable learners to see, touch, and manipulate them, demonstrating the dynamic nature of viruses and the conformational change that occurs when they interact with a living cell. Used both in-school and at science fairs and events, the large-scale models are complemented by maker-kit activities that engage children to creatively explore virus structure and function through reflective making.

Co-designed with over 400 students and 17 teachers at primary and secondary schools in West Yorkshire, we seek collaboration with children as expert users and engage them in an iterative design process. Here, we will share the design process, prototype evolution, and the learning gained through our approach to translate microscopic mechanical features of three viruses – poliovirus, coronavirus, and norovirus – into dynamic models that demonstrate virus structure and conformational change. Examples of maker-kit activities will be shared, alongside hands-on 3D models and design animations. Student, teacher, and science fair evaluation will be reported, reflecting on how the approach has helped develop the science capital of children and young people.

B305

Advancing academic integrity in microbiology education through a novel gamified online training module

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Abstract

Launched in June 2023, this project aims to develop a gamified online training module to bolster academic integrity in microbiology. Its primary goal is to enhance awareness of academic integrity, aid in navigating ethical dilemmas, and reduce academic misconduct. Spanning 21 months, the project's primary goal is to augment awareness and understanding of academic integrity, improve the ability to navigate ethical dilemmas and reduce instances of academic misconduct in the field. It is divided into four work packages (WPs). WP1 involved assembling a project team and conducting a needs analysis. Two focus groups have highlighted the necessity for a novel academic integrity training approach, contributing over 100 microbiology-specific ethical scenarios for gamification. WP2, currently in progress, focuses on creating the interactive module using these scenarios. A preliminary game version has been student-tested, with feedback informing ongoing refinements. WP3 and WP4 will focus on further testing and refining based on extensive student feedback, and evaluating impact. Key resources include faculty members, instructional designers, student representatives and a learning management system. Data collection methods, including surveys and focus groups, will assess the project's impact on student learning and success. Findings will be disseminated through various channels, including social networks, with artificial intelligence models evaluating stakeholder engagement. The project's microbiology focus, coupled with its scalability and transferability, underscores its potential for significant impact in promoting ethical practices in academic settings, especially in science disciplines and offers the opportunity to use gamification approaches with structured modules for example bioinformatics and microbiome analysis.

B306

Starting small: making microbiology accessible to young children

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Abstract

It can be challenging engaging young children about microbiology, microbes and microscopy, but it is important to capture the interest of these enthusiastic and enquiring minds at an early age. Examples of how microscopes with screens rather than eyepieces, Giant Microbes, inflatable dinosaurs and a magical microbiology top hat are used in activities and games to enthuse young children about microbiology and antimicrobial resistance (AMR) will be presented.

BLOCK B

Session : Microbial Physiology, Metabolism and Molecular Biology Forum

B309

Employing synthetic microbiology approaches to quantify signal output of bacterial two-component systems

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Abstract

The bacterial two-component system (TCS) consists of a protein, histidine kinase (HK), which interacts with the stimulus, and its cognate protein couple, response regulator (RR), which regulates a corresponding output. The TCS is ubiquitous in all bacterial species, thus, in the age of novel antibiotic discoveries, making it an interesting new drug target. However, many TCSs are not well understood enough to be incorporated in a high-throughput drug screening.

On the upside, one unique feature of TCS proteins is the conservation of its domains—signal transfer domains for HKs and signal receiving domains for RRs. Utilising this feature, a chassis created from a model TCS can be created to surrogate the output quantification of other TCSs, either via HK fusions or RR fusions. This study will focus on TCSs with membrane-bound HKs, therefore, two TCS chassis will be developed, *Bacillus subtilis* BceS/R for Gram-positive and *Escherichia coli* EnvZ/OmpR for Gram-negative.

Synthetic plasmids will be used to express chimeric proteins, fluorescence protein genes will be employed to track expression of proteins regulated by the RRs, BceR and OmpR, and FRET analysis will be used to track HK-RR interactions. Fine tuning techniques will be employed throughout the process, such as translation control using ribosome-binding site sequences to prevent overexpression of HKs, and aromatic tuning to restore sensory function of chimeric HKs. The expected outcomes of this study are the construction of fully functional combinations of HK and RR fusions, and the optimisation of a standardised chassis for TCS output quantification.

B310

CRISPR-based bacteriophage engineering reveals essentiality of anti-CRISPR-associated gene

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Abstract

Bacteriophages (phages) are virus that infect bacteria. To combat phage infection, bacteria possess various immune mechanisms, including CRISPR-Cas systems, which develop molecular memories of past infections to recognize and selectively destroy invader genomes. In response, phages have evolved anti-CRISPR (Acr) proteins, which are deployed upon infection and inactivate CRISPR-Cas systems to allow phage replication. The acr genes are often found accompanied by anti-CRISPR-associated (*aca*) genes. The function of Acas was found to be downregulation of acrs, but its biological significance in phage replication remains largely underexamined. Here, we established an endogenous CRISPR-Cas-based technique to engineer Pectobacterium carotovorum phage ZF40, a carrier of the *acrIF8*-*aca2* operon. We demonstrate that Aca2-mediated regulation is crucial to ZF40 replication. We applied a recombination and CRISPR counter-selection approach for ZF40. First, the ZF40 genome is modified through recombination with a plasmid containing regions of homology to the phage genome. Second, the host CRISPR-Cas system is programmed to target unmodified phages, resulting in selection for recombinant ZF40 phages. The deletion of *aca2* resulted in nonviability of ZF40 during the lytic cycle, demonstrating the essentiality of Aca2. Further ZF40 engineering demonstrated that the unregulated *acrIF8-aca2* promoter activity compromises ZF40 replication. In addition, AcrIF8 overexpression was shown toxic to host cells through conjugation efficiency assay. Altogether, we have established an efficient phage engineering platform and demonstrated that Aca2 protects ZF40 replication from toxicities associated with AcrIF8 overproduction and readthrough transcription from the *acrIF8-aca2* promoter.

B311

Investigating select substrates on a gut microbial community using an *ex vivo* fermentation model

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Abstract

The contribution of the gut microbiota to health and disease is becoming increasingly apparent due to developments in both DNA sequencing and cultivation techniques. There has been a lot of focus on enhancing the growth of desirable microbes, especially bifidobacteria and lactobacilli, using prebiotics, i.e., non-digestible food substrates which are selectively utilised by beneficial bacteria, and other dietary components. In this study, we employed an *ex vivo* colonic model to test a range of substrates, including a formulated beverage, in various combinations on a gut microbial community. *Ex vivo* models provide a reproducible, rapid, and inexpensive means of assessing the colonic microbiota. Samples were obtained at 0h and 24h to establish the impact of these substrates before and after fermentation. Shotgun metagenomic sequencing was applied to unravel the composition and functional changes over time. A combination of computational approaches were used including, species-level taxonomic classification, functional potential, the generation of metagenome-assembled genomes. Substrates differed in their impacts on the microbiota, but some consistent patterns were revealed, such as oligosaccharides supporting the increased abundance of bifidobacteria, specifically *Bifidobacterium adolescentis*, and lactobacilli members. Species-level alpha diversity was best maintained with lactose, a whey protein concentrate and xylo-oligosaccharide (XOS) combination, and XOS alone. This provides the basis for additional testing to determine the taxonomic and potential functional effects substrates have on the gut microbial community.

B312

Post-translational regulation of glucose metabolism in *Streptomyces*

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Abstract

Streptomyces are prolific producers of bioactive specialised metabolites which are biosynthesised from primary metabolic building blocks. Understanding the regulation of primary metabolism will enable engineering of metabolism to increase production of antibiotics and other clinically important metabolites from *Streptomyces*. One recently emerged regulatory mechanism in *Streptomyces* is post-translational modification (PTM) by crotonylation. The role of crotonylation as a post-translational modifier of glucose-kinase (Glk) and carbon metabolite repression (CCR) in *Streptomyces* metabolism is poorly understood. A previous study suggested the importance of crotonylation in the regulation of CCR in *Streptomyces roseosporus*, however, no evidence currently exists to suggest this mechanism occurs widely in *Streptomyces* species. Here we show that introducing the putative crotonylation and decrotonylation machinery from *Streptomyces coelicolor* into the industrial species *Streptomyces clavuligerus* alters antibiotic yield. We found that an over-expression of each of the components of the crotonylation/decrotonylation machinery resulted in a 1.5-fold increase in the yield of clavulanic acid and a 2-fold increase in bioactivity against *Micrococcus luteus*. Furthermore, the role crotonylation plays in PTM of *Streptomyces* metabolism was also determined in relation to the regulation of CCR in *S. coelicolor* and *S. clavuligerus* through knockout mutants and targeted metabolomics. This will allow for an enhanced understanding of the complex control mechanisms involved in *Streptomyces* primary and specialised metabolite production. Through this understanding there is potential to increase the yield of these vital natural products in industrial strains and make steps towards the identification and development of new clinically useful bioactive metabolites.

B313

The orthologous and paralogous nature of an Inc18 plasmid homologue that contains bacitracin-resistant genes in *Listeria monocytogenes*

Ogueri Nwaiwu, Helen Onyeaka

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Abstract

Listeria monocytogenes is still a food safety concern to food processors and the public in general due to its high mortality rate. Its detection in clinical settings is mainly due to the consumption of contaminated food. Food contamination can occur during the production, storage, or retail stages in the food chain and may cause significant economic losses to manufacturers. In the last decade, new atypical forms and species have been discovered due to advances in molecular biology. Recently, a tropical *L. monocytogenes* strain was found to contain an Inc18 plasmid sequence which had the presence of bacitracin-resistant genes. Plasmids can protect bacteria from harmful drugs and are notorious for the horizontal transfer of antimicrobial resistance genes. Also, the current global concern about antimicrobial resistance and the fact that bacitracin is a widely used topical antibiotic justified the investigation carried out. Up to 4725 plasmids and genomes were screened to ascertain the prevalence of the genes of interest among different bacteria genera. The strains screened were other Listeria species and several major genera of the phylum Bacillota (Firmicutes) like *Lactobacillus, Streptococcus, Enterococcus,* and *Staphylococcus*. The genera *Lactococcus, Pediococcus, Lactiplantibacillus, and Weissella* were included. Results showed sequence homology of 0-100% overall and only *L. monocytogenes* strains had over 92%. The bacitracin-resistant plasmid sequence was only present in some strains of *L. monocytogenes* and not up to 10 isolates had 99-100% homology. This indicates its paralogous nature and indicates that it is highly conserved at the moment, and not orthologous.

B314

Fattening up when starved - Carbonosome dynamics in non-growing Pseudomonas aeruginosa

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Abstract

Many non-sporulating bacteria, including the opportunistic pathogen Pseudomonas aeruginosa, endure niches which are hostile, competitive and nutrient-limited. These conditions elicit non-growing, antibiotic-tolerant phenotypes and restrict physiological activities to key preservative efforts. One such activity is the stockpiling of available carbon within "carbonosomes". However the dynamics, functionality and regulation of these complex intracellular inclusions remains mysterious.

Here, we show that P. aeruginosa readily produces carbonosomes under growth-prohibiting conditions. Utilising fluorescence-microscopy and flow cytometry, we developed assays to longitudinally quantify carbonosome dynamics. These methods revealed the temporal nature of carbonosome production and depolymerisation within non-growing cells. Dynamics were severely disrupted in mutants of established regulatory factors, which altered the size, number and intracellular positioning of carbonosomes. Furthermore, several under-characterised and un-annotated factors were also found to substantially dysregulate carbonosome dynamics.

By employing classical microbiological assays, carbonosome dynamics were found to impact motility and colony biofilm formation. Robust carbonosome dynamics were also found to correlate with bursts of protein synthesis induced by nutritional stress and conferred a fitness advantage during these conditions.

If future, we intend to longitudinally define the proteomic signature which accompanies carbonosome dynamics, and to uncover the interactome of regulatory factors. We hypothesise that carbonosomes are not innate carbon reserves, but play significant roles in the regulation of biosynthetic and energetic activities in non-growing cells. A deeper understanding of how these activities are regulated during non-growing states could ultimately unveil means for their disruption; presenting novel avenues for targeting tolerant populations or assisting in the strategic application of existing therapies.

B316

Bacterial Micro Compartments: A novel vaccine approach

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Abstract

Bacterial Micro Compartments (BMC) are icosohedral proteinous shells within certain species of Bacteria like *Clostridium autoethanogenum*, which when harnessed and genetically manipulated, can be used as a vaccine carrier or a drug delivery system. My studies have proved that BMCs can be made with just 3 vital proteins so that they can be smaller and increase their yield. One part of the study explored how these BMCs can be made into immunologically recognized vaccines for COVID-19 whereas another part of my study explored what happens when genes of similar function are hybridized - does it give stronger and higher yielding BMCs? A new type of BMCs? Bacterial phenotypes? These questions were answered using a plethora of Transmission Electronic Microscopic (TEM) images of the cross sections of these cells and their negative stains. The study also gave an insight into an interesting new phenotypical change within a bacterial species due to the presence of just 16 amino acids and the gene exhibited the same phenotype when a plasmid of the gene was introduced into *E.coli*! All these results gave rise to a very significant theory on the internal compartmentalization of BMCs and the role and timing of each protein and their significance. Though the BMCs are not a well-studied field in proteomics, results obtained from this study will contribute to the previously existing knowledge and pave the way to explore more of the BMCs' potential.

B318

Lost in Translation: Revisiting the *bldA* locus in *Streptomyces*

Jack W Stone, Iain S Hunter, Paul A Hoskisson

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Abstract

Streptomyces are responsible for the production of two-thirds of clinically relevant antimicrobial agents and understanding control of their biosynthesis will help address the challenge of antimicrobial resistance. The bald (*bld*) mutants in *Streptomyces* are blocked at an early stage of development and are unable to erect aerial hyphae, with many mutations in *bld* loci also pleiotropically blocking antimicrobial production. Mutations in the *bldA* locus, which encodes a rare leucyl-tRNA, result in a complete loss of morphological development and specialised metabolite production.

Previous work utilizing an RNA aptamer/fluorescent protein construct showed that transcription and translation of a TTA codon-containing gene in wild-type *Streptomyces* can be observed at an early stage in growth when $tRNA^{bldA}$ is not expressed. These data suggests that Wobble base-pairing (WBP) may occur at UUA codons when $tRNA^{bldA}$ is limiting. The CAA tRNA (UUG codon) is the closest anticodon that could be mismatched with the UAA tRNA anticodon (UUA codon). Here, we discuss the sequencing the original *bldA* mutant in *S. coelicolor* (J1700 strain) that was isolated in the 1970s and the use of complementation studies to further understand the potential role of WBP in *bldA* relating to specialised metabolite production and sporulation. Testing the hypothesis that the CAA tRNA could complement a *bldA* genetic lesions through providing a WBP match for the UUA codon, and attempting to answering the question of why translation of the TTA codon could be observed even in the absence of the UAA tRNA.

B320

Unbiassed search for pyocin S3 receptor on target cells by random mutagenesis

Samar Alonayzan, Cemile Aksoy, Faisal Albaqami, Kim R Hardie, Christopher Penfold

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Abstract

The life-threatening opportunistic multidrug-resistant pathogen *Pseudomonas aeruginosa* is commonly implicated in hospital-acquired infections. Due to the limitations of the current antibiotics and the challenges in treating *P. aeruginosa* infections, novel antibiotics or therapeutic alternatives are needed urgently. This research aims to investigate pyocin S3 (PyoS3) to develop novel treatments for *Pseudomonas* infection. PyoS3 is a potent bacteriocin produced by *P. aeruginosa* that kills certain sensitive *P. aeruginosa* strains, but the mechanism is poorly understood. To provide knowledge on the mechanism of PyoS3 uptake and activity, a random mutagenesis approach was taken to identify potential genes involved in importing PyoS3 into a sensitive cell. A transposon library of the sensitive clinical strain PSA892 was generated and screened for its response to the purified PyoS3 to identify defective resistant mutants for a gene involved in PyoS3 uptake. More than 7500 mutants were screened for their sensitivity to PyoS3, and several mutants were identified that were either no longer killed by or showed increased sensitivity to PyoS3. DNA sequencing revealed that one of them had a transposon inserted into the *APH(3')-IIb* gene which is related to aminoglycoside 3'-phosphotransferase. Although a defined mutant deficient in *APH(3')-IIb* revealed that *aph* is not involved in PyoS3 uptake, the mechanistic role of other candidate proteins that were will be reported. Detecting potential genes involved in the uptake of PyoS3 is the first step to uncovering the killing mechanism as a basis for developing it as a novel therapeutic.

B322

A novel Arthrobacter ilicis subspecies takes on xanthan degradation: physiology, insights, and enzymes

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Abstract

Environmental samples enriched with xanthan gum led to the discovery and isolation of a novel subspecies of Arthrobacter ilicis which carries a previously unseen plasmid with a xanthan degradation gene region. This bacterial organism is thus a new xanthan degrading microbe discovered in Bielefeld, Germany, and is the first from the Arthrobactrer genus to degrade xanthan. Additionally, growth experiments were able to determine both the pH and temperature optimum (pH 7 and 28-30° C) of this novel xanthan degrading subspecies.

A comparison of intra- and extracellular proteins, using an LC-MS approach to investigate bacterial cultures of this species grown in glucose or xanthan, exposed significant differences in protein abundance. Among the most highly upregulated proteins seen when feeding on xanthan were the genes from the xanthan degradation gene region coding for the PL8 xanthan lyase, GH38, GH3, and GH9, which together provide for a prospective xanthan degradation pathway.

B324

The ZomB helicase: a novel regulator of pneumolysin expression in *S. pneumoniae*

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Abstract

Streptococcus pneumoniae (*S. pneumoniae*) is a bacterial pathogen responsible for several diseases, such as otitis, pneumonia, meningitis and bacteremia, causing significant morbidity and mortality worldwide, despite vaccine availability. The pore-forming toxin pneumolysin is one of the most important virulence factors for this pathogen and it has multiple roles during infection. However, very little is known about the regulation of the expression of this toxin. We performed a genome-wide association study (GWAS) to identify genomic signatures associated with *S. pneumoniae* haemolytic activity, a proxy measurement for pneumolysin production. The GWAS highlighted four SNPs associated with haemolysis encoded in a previously uncharacterized gene, later named *zomB*. *zomB* is located on an integrative conjugative element specific to the pandemic SPN23F ST81 lineage. It encodes for a UvrD-like helicase, deletion of which causes downregulation of pneumolysin transcription. A pulldown study was performed to find ZomB's protein partners and clarify how it could affect pneumolysin expression. The pulldown and a bacterial-two hybrid assay showed that ZomB interacts with the CiaR regulator which controls cell wall biosynthesis and competence. However, CiaR doesn't affect pneumolysin production. The pulldown also showed several other potential protein partners that could interact with ZomB to exert a transcriptional regulation on pneumolysin, including a DNA methylase, an adenylate cyclase and an exoribonuclease. The role of these proteins is currently being investigated. Overall, this study uncovers ZomB as a novel regulator of transcription of the key toxin pneumolysin and highlights unexpected ways in which bacterial helicases can regulate gene expression.

B325

Characterising the *Orientia tsutsugamushi* infection cycle: how do intracellular and extracellular bacteria differ?

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Abstract

Orientia tsutsugamushi is an obligate intracellular bacterium which causes scrub typhus, a mite-borne infection that is a leading cause of non-malarial febrile illness in South and South East Asia. The life-cycle of *Orientia tsutsugamushi* involves differentiation of the bacterium from the intracellular state localised in a perinuclear localised microcolony, to the extracellular state which buds from the surface of cells in a virus-like manner. Here we characterise the intracellular and extracellular state through immunofluorescence microscopy, RNA-SCOPE and proteomic analysis. We identify proteins which differ in expression between intracellular and extracellular bacteria.

B327

Pore forming toxins of the Type VI secretion system: Knowing when to hold them and knowing how to fold them

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Abstract

The Type VI secretion system (T6SS) is a dynamic macromolecular structure that promotes contact dependent killing of competitor microorganisms through the injection of toxic effector proteins. While several classes of soluble, enzymatic effector have been described in detail, characterising effectors that target the cell membrane has proved much more challenging. We have employed bacterial co-culture assays and voltage clamp electrophysiology to characterise the activity of a novel membrane associated effector from *Serratia marcescens*. The effector is a potent antibacterial toxin with a broad target range that promotes killing through a loss of membrane polarity. Transposon insertion sequencing studies revealed no mutants with specific resistance to this toxin but instead identified two gene disruption mutants with global resistance to T6SS effector delivery. Together our data provide insights into the function of pore forming toxins, the target range of different T6SS effectors and the global T6SS resistance mechanisms that can arise under nominal selective pressure.

B328

EnvR is a potent repressor of *acrAB* transcription in Salmonella

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Abstract

RND efflux pumps confer multidrug resistance (MDR) in Gram-negative bacteria by pumping out clinically-relevant antibiotics. From the TetR family of transcriptional regulators (TFTRs), EnvR is an effective repressor of *acrB* transcription, the primary RND pump in Enterobacteriaceae. However, expression of EnvR is constitutively suppressed due to H-NS silencing. To determine its effects upon the efflux activity of AcrAB-TolC, *Salmonella enterica* serovar Typhimurium SL1344 was used to construct a strain which overexpressed EnvR from the chromosome via homologous recombination. Under the control of a constitutively active promoter, EnvR is produced at high levels. Due to its regulatory nature and high affinity for the *acrAB* promoter, *acrB* expression is effectively reduced. The phenotypic effect of this transcriptional regulation was observed by measuring the rate of compound removal from within bacterial cells, where EnvR overexpression led to significantly lower AcrB efflux activity compared to wild-type SL1344. The minimum inhibitory concentration (MIC) values of this strain also showed increased antimicrobial susceptibility against an array of substrates. Additionally, RNA-sequencing revealed that the global transcriptomic response to EnvR overexpression was distinct from that of *acrB* inactivation, and provided candidate genes for further investigation. Taken together, this work indicates that EnvR regulation of AcrB expression can significantly render bacteria avirulent and susceptible to antibiotics. Exploiting the functions of regulators like EnvR seems to be a promising avenue to combat antibiotic resistance.

B329

Modulation of morphological features of *P. aeruginosa* colony biofilm by amino acids

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Abstract

Pseudomonas aeruginosa, a Gram-negative pathogen that is ubiquitous in the environment displays various lifestyles ranging from individual to social communities such as biofilm and swarm. Colony biofilm in *P. aeruginosa* is a quorum sensing dependent phenomena, consisting of mostly sessile bacteria encased in a matrix of extracellular polymeric substances (EPS) containing *pel*, *psl* polysaccharides and alginates. Colony biofilm on agar plates stained with Congo-Red and Coomassie Brilliant Blue provides features such as coloration and definitive morphological features, such as wrinkles. It is believed that high nutrient concentration promotes biofilm formation by inducing secondary messenger cyclic di-GMP concentration. To understand the effect of nitrogen sources on biofilm formation, we asked whether specific amino acids in the growth media could impact biofilm formation in *P. aeruginosa*. We used M9 minimal media plates containing 0.5% casamino acids (CAA) for the study of colony biofilms. In test experiments, CAA were replaced by each of the 20 amino acids one at a time. We observed that each amino acid tends to affect the morphological features of the biofilms, like coloration, wrinkling, and growth of the biofilms, when imaged in a time-dependent manner. This is an ongoing study to see how different nutritional cues affect motility and growth of *Pseudomonas aeruginosa*. Analysing biological fluids such as lung lavage in terms of amino acid availability can provide insights into biofilm formation and pathogenesis, opening further avenues of prevention and control.

B330

A novel chronic wound infection model to study *P. aeruginosa* virulence factor AaaA.

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Abstract

Pseudomonas aeruginosa poses a formidable challenge in healthcare settings due to its intrinsic multi-drug resistance, leading to persistent infections in immunocompromised individuals and a significant global disease burden. This is particularly evident in the context of cystic fibrosis, where *P. aeruginosa* is a primary cause of fatal lung infections, and in chronic wound infections where it frequently coexists with other bacteria. This research focuses on unravelling the functional intricacies of a key virulence factor, the arginine-specific aminopeptidase of *P. aeruginosa* (AaaA), which is crucial for the establishment and persistence of chronic infections. Understanding AaaA's role is pivotal, as it could potentially serve as a target for vaccines or antimicrobials. To mimic the host infection site realistically, a synthetic chronic wound model incorporating *Staphylococcus aureus* alongside *P. aeruginosa* was developed. Quantification of AaaA activity in this model demonstrated a time-dependent increase, while a mutant lacking *aaaA* exhibited significantly reduced L-arginine cleavage within just 2 days. Confocal microscopy highlighted distinct differences in biofilm formation between the *aaaA*-defective mutant and the wild type after 4 days. These findings strongly support the hypothesis that AaaA plays a crucial role in biofilm formation, explaining its significance in chronic compared to acute infections. Ongoing enhancements to the infection model involve the incorporation of clinical isolates of *P. aeruginosa* (along with their *aaaA*-defective mutants) in combination with co-isolated *S. aureus*. This enhanced model aims to provide insights into how AaaA could be targeted effectively in a polymicrobial context, offering valuable implications for the development of antimicrobial strategies.

B331

Construction of a Recombinant Lactic Acid Bacteria Expressing SARS-CoV-2 S1 Receptor-Binding Domain for Mucosal Vaccine Development

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Abstract

Background: The main site of transmission of SARS-CoV-2 is mucosal surfaces. To fight against the infectious disease, vaccines that can produce systemic immunity as well as secretory IgA are highly desirable. Mucosal vaccines that trigger production of IgA could prevent infection and population transmission. Lactic acid bacteria (LAB) are excellent options for the development of safe production and delivery systems of protein antigens.

Objective: The aim of this study was to develop a mucosal vaccine by applying LAB as a bacterial vector for expressing S1 receptor-binding domain (RBD) of SARS-CoV-2 spike protein.

Methods: To construct a recombinant LAB expressing SARS-CoV-2 spike S1 RBD, pNZ8148 *Lactococcus lactis* expression system was used. The S1 RBD was cloned into the pNZ8148 vector along with poly-γ-glutamic acid synthetase A (pgsA) as applied to display exogenous proteins on the surface of LAB. *Escherichia coli* MC1061 was used as a primary host strain. The newly engineered vector was further transformed into *L. lactis* NZ9000 used as a host for S1 protein expression.

Results: Our confirmations revealed that S1 was cloned into the pNZ8148 vector. DNA sequencing showed the length of pgsA ligated with S1 of 1862 bp. A digestion method confirmed that S1 was correctly inserted into the vector cloning site. This construct was successfully transformed into the *L. lactis* NZ9000. Therefore, this *L. lactis* will be confirmed for S1 expression on its cell surface as an antigen display, and the ability to induce systemic immunity as well as secretory IgA in a mice model.

B332

Molecular Evidence for Occurrence of Heavy Metal and Antibiotic Resistance Genes Among Predominant Metal Tolerant Pseudomonas sp. and Serratia sp. Prevalent in the Teesta River

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Abstract

Riverine ecosystems polluted by pharmaceutical and metal industries are potential incubators of bacteria with dual resistance to heavy metals and antibiotics. The processes of co-resistance and cross resistance that empower bacteria to negotiate these challenges, strongly endorse dangers of antibiotic resistance generated by metal stress. Therefore, investigation into the molecular evidence of heavy metal and antibiotic resistance genes was the prime focus of this study. The selected *Pseudomonas* and *Serratia* species isolates evinced by their minimum inhibitory concentration and multiple antibiotic resistance (MAR) index showed significant heavy metal tolerance and multi-antibiotic resistance capability, respectively. Consequently, isolates with higher tolerance for the most toxic metal cadmium evinced high MAR index value (0.53 for *Pseudomonas* sp., and 0.46 for *Serratia* sp.) in the present investigation. Metal tolerance genes belonging to PIB- type and resistance nodulation division family of proteins were evident in these isolates. The antibiotic resistance genes like *mexB*, *mexF* and *mexY* occurred in *Pseudomonas* isolates while *sdeB* genes were present in *Serratia* isolates. Phylogenetic incongruency and GC composition analysis of PIB-type genes suggested that some of these isolates had acquired resistance through horizontal gene transfer (HGT). Therefore, the Teesta River has become a reservoir for resistant gene exchange or movement via selective pressure exerted by metals and antibiotics. The resultant adaptive mechanisms and altered phenotypes are potential tools to track metal tolerant strains with clinically significant antibiotic resistance traits.

B333

Exploring the impact of air pollution exposure on bacterial behaviour and epithelial cell interaction

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Abstract

Air pollution is the single largest environmental health risk worldwide. Particulate matter (PM) air pollution is caused by fossil fuel combustion and vehicle motion, breaking and tyre wear. It has been shown that exposure to PM can cause increased levels of respiratory disease, including exacerbations of chronic obstructive pulmonary disease (COPD). COPD is frequently associated with *Haemophilus influenzae* infection, but whether these infections are a cause or consequence of COPD exacerbation remains unclear. Our previous publications showed that as well as damaging the host, PM has a direct impact on pathogenic respiratory bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Our current research shows that exposure of non-typeable *H. influenzae* 375 (NTHi375) to black carbon (BC), a major component of PM, prior to infection increases their ability to adhere to A549 respiratory epithelial cells. This suggests that the frequency of bacterial infection induced COPD exacerbation may be altered in patients from highly polluted areas, and therefore a potential link between two major causes of COPD exacerbation. RNA-seq analysis of NTHi375 cultures showed that BC significantly impacts NTHi375 iron homeostasis. Fur (ferric uptake regulator) regulated genes including *hitA*, *hxuC*, *tbp1/2*, and *tonB* are all upregulated in response to BC exposure, while iron storage proteins FtnA and FtnB are downregulated. In addition to this, *hpf*, a gene encoding the ABC-metal transporter protein F, known to facilitate A549 binding, is upregulated in response to BC exposure. These data provide evidence of a mechanistic link between particulate PM induced colonisation and iron homeostasis in NTHi375.

B334

Revealing the coregulation of key virulence machines in *Pseudomonas aeruginosa*

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Abstract

As a notorious human pathogen, *Pseudomonas aeruginosa* still causes countless in-hospital infections each year with huge costs. To understand its role as the pathogen, efforts have been made to discover the innate regulation of virulence systems. In this project, we specifically focused on Type III Secretion System(T3SS), which is used for anti-eukaryotic cell killing, and Type VI Secretion System(T6SS), for inter-bacterial competition. By designing novel reporters from different perspectives of the virulence activity, we emphasized the involvement of the key second messenger, c-di-GMP in the interplay of the virulence network and compared the discovered link with other model pathogens. Aside from the populational discoveries, for the first time we tried to directly visualize the crosstalk of the virulence systems on a single-cell level using microscopy techniques and thus constructed a vivid picture of the division of labor on the behavior of virulence, possibly leading to a finely-tuned virulence dynamics model during the course of complex infection. Combining classic and newly designed molecular biology methods, the current findings and ongoing research can greatly help understand *Pseudomonas aeruginosa* as a pathogen and provide insights in blocking *Pseudomonas* infections.

B335

The Role of Bacterial Membrane Potential in Antibiotic Persistence

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Abstract

The membrane potential of bacteria facilitates essential physiological processes, including ATP synthesis, motility, and cell division. Bacterial membrane potential has also been suggested to be associated with antibiotic persisters -- subpopulations of genetically sensitive bacterial cells that are capable of surviving antibiotic treatment as a result of phenotypic dormancy, which can then re-grow following the end of antibiotic treatment. Antibiotic persisters of different pathogens have been associated with a wide range of recurrent and chronic infections; additionally, it has been suggested that persistence may accelerate the emergence of genetic antibiotic resistance within these strains. However, research into the mechanisms of bacterial persistence and development of anti-persister therapeutics is limited by the inability to identify persisters prior to antibiotic exposure and re-growth, after which the growing cells are no longer in the persister phenotypic state. Therefore, we utilised genetically encoded bacterial membrane potential sensors in combination with a microfluidics-microscopy platform to study the membrane potential dynamics of single cells before, during, and after antibiotic treatment, to identify if bacterial membrane potential is a reliable indicator for the persister phenotypic state.

B336

Probing crosstalk between peptidoglycan and energy transduction during bacterial cell division

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Abstract

Cell wall remodelling during cytokinesis is a key event in bacterial cell division and as a result a viable target for the development of new antibiotics to combat the rise in AMR. During this event, Gram-negative bacteria coordinate the simultaneous biogenesis and separation of their tripartite cell envelope, composed of the inner membrane (IM), peptidoglycan cell wall (PG) and outer membrane (OM). How bacterial cells synchronise separation of the cell wall while invaginating the OM is poorly understood. Remodelling septal PG requires coordination of two opposing activities: PG synthesis, performed by penicillin binding proteins (PBPs) such as FtsI, and PG cleavage performed by amidases and lytic transglycosylases (LTs). Recent evidence points to these systems being regulated by the energy-transducing Tol machinery that spans the two membranes of the cell envelope. Here, we examine the effects of individual *tol* operon gene deletions on *Escherichia coli* cell wall architecture. Using electron and fluorescent microscopy, we show that there are fundamental differences in sacculi structure of individual *tol* knock-outs as well as sensitivity towards PG synthesis inhibitors. In contrast to literature reports that *tol* mutants increase antibiotic sensitivity, we find that loss of TolA, which transduces mechanical energy to the OM, confers resistance to antibiotics that specifically target FtsI at division septa. Our results support a model in which the energy-transducing Tol machinery, in addition to its role in stabilising the OM at division also coordinates the enzymatic activities necessary for completion of daughter cell separation.

B337

Determining the cellular location of multicopper oxidase Mco in *Staphylococcus aureus.*

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Abstract

Despite being a member of the nasal microbiome in around 30% of the population, *Staphylococcus aureus* can cause life-threatening infections such as sepsis and pneumonia. The *copB-mco* copper hypertolerance operon is found in clonal complexes (CCs) of *Staphylococcus aureus* associated with healthcare acquired infection (CC22) and nasal colonisation (CC30). The operon encodes the CopB P_{1B}-type ATPase and Mco multicopper oxidase. Mco converts copper from its more harmful form Cu(I) to Cu(II), which is less damaging to the bacterial cell due to having decreased redox potential. The cellular location of Mco within *S. aureus* is yet to be identified, as its homologs within Gram-negative bacteria are periplasmic. We generated the pCL55::*copB-mco*F construct, which encodes the full *copB-mco* locus, with the Mco protein tagged with a 3xFLAG tag. Growth kinetics data of Newman *spa sbi* pCL55::*copB-mco*F and Newman *spa sbi* pCL55::*mco*F suggest that acquisition of the pCL55::*copB-mco*F plasmid confers copper hypertolerance, while loss of *copB* causes a loss of copper hypertolerance, suggesting that both *copB* and *mco* are required for a copper hypertolerant phenotype. Western blotting using anti-FLAG IgG suggested that Mco is present in membrane fractions generated from *S. aureus*. Structured illumination microscopy using an anti-FLAG IgG along with Nile Red membrane staining shows that Mco is membrane located both in the strain with the full *copB-mco* locus and the *copB* mutant. In summary these data shed new light on the cellular location of the multicopper oxidase Mco in *S. aureus*.

B338

Adaptive metabolic rewiring in maintaining redox homeostasis in NADH dehydrogenase deficient Escherichia coli

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Abstract

Microbes adapt and thrive in diverse environmental niches by rewiring their transcriptional and translational processes. This adaptability requires an energy metabolism that can support the specific demands of each condition. These energy-intensive processes are supported by the aerobic electron transport system (ETS), which is the most efficient and dynamic process for generating energy. The dynamism arises due to the branching in bacterial ETS.

We are investigating the branching at the electron entry point in ETS using the NADH dehydrogenases NDH-I and NDH-II mutants in Escherichia coli. The NDH-I is a large multisubunit complex mediating NADH oxidation coupled with proton translocation. NDH-II is a ten-times smaller, single-unit protein mediating only the oxidation of NADH but is preferred in aerobic respiration as it has a higher turnover for generating NAD+, the main cellular oxidant. Moreover, the absence of NDH-II in human mitochondria makes it a desirable antibacterial target.

We performed laboratory evolution to understand the stable effects of NDH-II perturbations in E. coli. Surprisingly, the growth defect due to the lack of NDH-II was compensated by a decrease in the activity of complex II, an enzyme which links TCA to ETS. Systems-level analysis using genome-scale metabolic models indicates that the cells reduced flux through NADH-producing reactions while maintaining the overall flux through central carbon metabolism. This flux rewiring enables the maintenance of redox homeostasis through the increased expression of low turnover NDH-I. We are currently examining the tradeoffs underlying complex I and complex II discord in physiologically relevant systems.

B339

Characterising novel lipase toxins of the *Staphylococcus aureus* Type VII Secretion System

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Abstract

The Type VII Secretion System (T7SS) of Firmicutes is used to export effector proteins across the cell envelope, including toxins. Recently, in the human and livestock pathogen *Staphylococcus aureus*, T7SS substrates have been identified with antibacterial toxicity. These toxin genes appear alongside those which encode for specific immunity proteins, which form complexes to protect against self-intoxication. Additionally, the discovery of widespread T7SS immunity genes within strains lacking the toxins themselves points to this likely antibacterial function, and a dynamic interbacterial evolutionary arms-race involving novel toxins and immunity defences. Recent bioinformatics analysis has identified two novel families of putative lipase toxins of the *S. aureus* T7SS repertoire. They share an unusual domain structure and predicted lipase activity, but are otherwise very unique genetically.

Utilising a split-nanoluciferase assay, T7SS-dependent secretion of one of the lipase toxins has been confirmed. Additionally, both toxins have been expressed recombinantly in *Escherichia coli* for bacterial two-hybrid analysis alongside their putative partners. This has revealed interesting information about their interactions *in vivo.* Further work will involve biochemical characterisation of the toxins to confirm their predicted lipase activity, as well as toxicity assays in vulnerable *S. aureus* “prey” mutants lacking immunity genes. Investigation of these novel families and their toxicity, function and relevance to interbacterial competition will reveal more about the dynamic role the T7SS and its repertoire of diverse effectors plays in the biology of *S. aureus.*

B340

Proteomic response of CTX-M-15 producing *Escherichia coli* in cefotaxime

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Abstract

Background: The enzyme CTX-M-15*,* an extended spectrum beta lactamase, has potent hydrolytic activity against the third generation cephalosporin cefotaxime. By using Mass Spectrometry, the proteomic response of a CTX-M-15 producing strain of *Escherichia coli* to cefotaxime can be studied, comparing to a susceptible strain of the same species.

Methods: A cefotaxime resistant and CTX-M-15 producing strain *E. coli* DSM 22664, and a cefotaxime susceptible strain *E. coli* MG1655, were grown in x2 their MIC of cefotaxime for 2 hours. The proteins were extracted and digested with trypsin, before being analysed on a Q-Exactive Mass Spectrometer.

Results: Proteins increased and decreased in *E. coli* DSM 22664, compared to *E. coli* MG1655 were analysed using Max Quant and Perseus Software. Proteins increased in the resistant strain included ribosomal assembly proteins such as 50S ribosomal protein L16, and 30S ribosomal protein S10 as well as cell wall synthesis proteins such as D-alanyl-D-alanine endopeptidase and penicillin-binding protein 1B. Proteins increased in the susceptible strain, and thus decreased in the resistant strain, included shock and stress proteins such as acid stress chaperone HdeB, and cold shock-like protein CspE,

Conclusion: By studying the proteomic response of a resistant and susceptible *E. coli* to cefotaxime, key proteins and pathways can be identified, which could be potential targets for novel antimicrobial treatments.

B341

A new post-transcriptional regulator of virulence and environmental adaptation in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen, posing a major threat in the recovery of hospitalised and immunocompromised patients. P. aeruginosa is able to establish antibiotic-resistant long-term infections due to its metabolic versatility and wide array of virulence factors. Significant contributors to establishment of infection by this organism are the three quorum sensing systems (rhl/las/pqs) together with a broad range of small non-coding RNAs (sRNAs) aiding to swift adaptation to new environments. Here, we investigated the role of a sRNA encoded within the promoter of the pqsABCDE operon of the pqs system, which we named PqsX. Despite its genetic connection to the pqsA promoter, PqsX was not shown to interfere with the regulation of the pqs system. By implementing in vivo proximity ligation and sequencing (GRIL-Seq), it was uncovered that PqsX is involved in multiple virulence pathways and has preferential binding at the 3' end of RNA targets, unlike most well-studied sRNAs. RNA-Seq revealed a strong connection of this sRNA to iron regulation which was further supported by changes in pyoverdine production in a pqsX mutant. The involvement of PqsX in diverse metabolic and virulence pathways emphasises the multifaceted role of sRNAs in regulating virulence. These RNAs play a crucial role in fine-tuning genetic response to environmental changes, considerably impacting the pathogenesis of P. aeruginosa.

B343

Understanding *Escherichia coli* persister diversity to improve treatment strategies

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Abstract

Many bacterial infections cannot be cured, even when caused by a pathogen that is not resistant to antibiotics. Antibiotic tolerance and persistence enable bacterial cells to survive transient exposure to antibiotics at concentrations that would otherwise be lethal, often by entering a slow-growing or dormant state. After treatment ends these cells can revive and regrow, leading to recurrent and chronic infections. Developing new strategies to eradicate persisters and reduce antibiotic treatment failures requires better understanding of how the physiology of diverse persister mutants affect their susceptibility to different antibiotic classes.

In this study, we tested how different molecular triggers of a high-persister phenotype affect *Escherichia coli's* ability to survive antibiotic treatment, describing their antibiotic tolerance profile across clinically relevant drugs and aiming to identify their Achilles' heel. We screened a collection of *E. coli* persister mutants against a diverse panel of antibiotics and drug combinations, using high-throughput phenotyping. While persister cells are typically thought to be tolerant to multiple classes of antibiotics, our results suggest that this tolerance is not uniform and depends on the specific cell physiology underlying the persister state. For example, some persister strains seem tolerant to several members of the of the β-lactam and fluoroquinolone antibiotic families, but only displayed minimal cross-tolerance to aminoglycosides or phosphonic antibiotics, which remain potential treatment options. Ultimately, the knowledge gained from this study will reveal the most promising treatment for infections caused by tolerant and persistent bacteria, opening the door to developing strategies to target specific persister types.

B346

Understanding the functional role of respiratory oxidase enzymes in the bacterial pathogen *Enterococcus faecalis*

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Abstract

Enterococcus faecalis is a common commensal of the gastro-intestinal tract of humans and animals. However, they also opportunistically cause serious nosocomial infections and can disseminate into a wide range of body sites. This ability combined with their high degree of intrinsic and acquired antimicrobial resistance (AMR) makes enterococci a high-risk pathogen. The electron transport chain (ETC) of many key AMR pathogens is being exploited for novel drug targets, but not in *E. faecalis* as the ETC is entirely uncharacterized. We have previously observed inverse regulation of the key respiratory oxidases cytochrome *bd* (CydAB) and the NADH oxidase (Nox) in response to antimicrobial challenge. Therefore, we aimed to determine the functional role of these respiratory oxidases in *E. faecalis*.

I have generated a set of isogenic knockout mutant strains for each component of the ETC and Nox. These strains have been characterized for their functional role in growth, survival, and regulation under different physiologically conditions. Phenotypic characterization has revealed two differential roles for CydAB and Nox. Nox is the major consumer of oxygen in *E. faecalis*, with an 87% reduction in oxygen consumption in a *Δnox* deletion mutant despite no effect on bacterial growth. This suggests that Nox functions as an oxygen sink to generate NAD^+ for anaerobic metabolism during nutrient rich environments. However, when NADH is limited the more efficient ETC compensates and *E. faecalis* transitions to aerobic metabolism. Together this highlights the high degree of metabolic flexibility that may aid in niche adaptation and therefore the pathology of *E. faecalis.*

B348

Tug of war for iron between *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

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Abstract

Competition for resources is one of the major drivers for the evolution and retention of new traits in microbial communities. Quorum sensing-dependent traits of opportunistic human pathogen *Pseudomonas aeruginosa* allow it to survive and thrive in nature. Here, we report a unique surfactant-driven pushing mechanism that *P. aeruginosa* employs specifically against *Klebsiella pneumoniae*. The pushing is accomplished in a manner that is dependent on nutrient limitation and quorum sensing. We find that *P. aeruginosa* employs neither proteases nor toxic secondary metabolites against *K. pneumoniae*. Rhamnolipid biosurfactant appears to be the only factor required to displace Klebsiella effectively. Both rhamnolipid production and the pushing ability of *P. aeruginosa* are suppressed by iron supplementation. We show that both these bacteria produce several siderophores in a minimal medium and rapidly deplete iron. Under these conditions, *P. aeruginosa* pushes Klebsiella away from the substratum using rhamnolipid, reducing the competition for iron. Our study describes a unique quorum and iron-responsive mechanism in *P. aeruginosa* to support its own growth during resource competition.

B349

Analysis of Antimicrobial Resistance and Virulence in Klebsiella pneumoniae Derived from Different Geographical Locations

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Abstract

Klebsiella pneumoniae is increasingly becoming a threat to human health, primarily due to the emergence of many multidrug-resistant strains and hypervirulent strains. It can occupy many different ecological niches, but the aspects of its infection of hosts other than humans have not yet been elucidated. Understanding correlations between geographic location and the acquisition of antimicrobial resistance and virulence factors is imperative for clinicians to develop effective treatment methodologies. Here we present a series of comprehensive analyses on the frequencies of the presence of AMR genes conferring resistance to 14 different antibiotic classes in a diverse array of K. pneumoniae strains, possession of all identified AMR determinants, and quantification of their corresponding virulence levels. Such analyses enabled the identification of the most common modes of acquiring AMR and virulence factors amongst isolates recorded in the dataset. Additional analyses were performed to provide insights into potential molecular mechanisms underlying the dissemination of integrative and conjugative elements encoding yersiniabactin and the role of hypermucoviscosity in hypervirulence. Furthermore, analyses were performed to find potential correlations between geographic location, antimicrobial resistance, and virulence. The generated phylogenetic trees enabled the identification of putative outbreaks. In the dataset of K. pneumoniae genomes from NCBI, many AMR determinants were prevalent, whereas high virulence scores were uncommon. The two outbreaks identified were localized to specific hospital institutions and composed of isolates that induced nosocomial infections. Many K. pneumoniae strains have rendered several antibiotics ineffective and disseminated efficiently in variable environments, a phenomenon illustrated clearly through the statistical analyses performed.

B350

Not so negative after all? How a repressor of the Type III Secretion System in Pseudomonas syringae regulates bacterial processes to improve virulence.

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Abstract

Pseudomonas syringae pathovars are major bacterial plant pathogens that utilize the Type III Secretion System (T3SS) to effectively deliver effector proteins to the plant cells. Elegant orchestration of this molecular machine is required for the optimal deployment of system components, when required, while avoiding unnecessary expenditure. The majority of T3SS structural and regulatory genes are co-localized in “pathogenicity islands” and most upstream of the cascade of interactions that drive T3SS activation, HrpV is found. Effector protein genes are characterized by a specialized promoter that is recognized by the T3SS-specific *σ* factor HrpL, itself transcribed by a σ^{54}-bound RNA polymerase that is activated by the HrpR/HrpS AAA proteins, in turn downregulated by the direct interaction of HrpS with HrpV. We have recently found that HrpV, while long considered mainly a repressor of T3SS, can also act as a co-chaperone to the gatekeeper protein HrpJ, directly linking T3SS protein expression to secretion. Moreover, Δ*hrpV* knockout mutants exhibit reduced disease severity in plant tissue. Phenotypic and whole transcriptome analysis of Δ*hrpV* mutants reveals that HrpV is involved and co-regulates other genes, e.g. related to aminoacid transport and metabolism, highlighting it as a pivotal coordinator of the interplay between T3SS and bacterial processes for the efficient infiltration to plant tissue and facilitation of virulence.

B351

Transcriptomic profiling of phage-host interactions between the LES prophages and Pseudomonas aeruginosa PAO1 under single and co-infection

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Abstract

Pseudomonas aeruginosa is an important opportunistic Gram-negative bacterium causing nosocomial infections. Among this species, the Liverpool Epidemic Strain (LES), a major cause of mortality and morbidity in cystic fibrosis patients, harbours five prophages associated with increased fitness and survival in infection models. However, little is known about the LES prophage interactions with the lysogen and other prophages. Here, we created single-, double- and triple-lysogen variants of the well-characterised *P. aeruginosa* strain PAO1 after infection of combinations of the LES prophages Φ2, Φ3 and Φ4. We applied transcriptomics to map the differential expression gene profiles of the LES prophages and predict the metabolic and physiological changes induced by these phages under inducing and non-inducing conditions. Among the different PAO1 lysogens, we identified ~20% of host differentially expressed genes (DEGs). Many of these DEGs were associated with several amino acid-related metabolic pathways such as L-arginine degradation, denitrification and nitrogen utilisation, secondary metabolic pathways such as alginate and polyhydroxyalkanoate synthesis, cell wall maintenance, virulence factors, motility, quorum sensing, biofilm formation and drug pumps. All these findings will be crucial for the role of temperate phages and for improving our understanding of host-phage coevolution.

B353

Multi-tier intertwined regulation of siderophore synthesis of *Shewanella oneidensis* by multiple regulatory systems

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Abstract

The siderophore-dependent iron uptake pathway is essential for *Shewanella oneidensis* whose iron requirement is high, yet the regulation of primary siderophore putrebactin synthesis remains largely unexplored. Here, we identified two positive regulators of the putrebactin synthesis, BarA, and SsoR, and found they operate within distinct pathways. BarA/UvrY two-component system regulates putrebactin synthesis operon *pub* post-transcriptionally via the Csr regulatory cascade. Specifically, UvrY activates the transcription of small RNAs, CsrB1, and CsrB1, which counteract CsrA's translational repression. The RNA-binding protein CsrA binds to the 5′ untranslated region of the *pub* transcript to repress the translation. Intriguingly, the orphan response regulator SsoR activates the transcription in a phosphorylation-independent manner, a departure from the conventional activation mechanisms observed in typical response regulators. Bioinformatics analysis highlights SsoR's uniqueness in phylogeny and conformational distribution. Combining AlphaFold2 and Molecular dynamics simulations, we observed conformational distribution differences of OmpR family members' structures and accessed the impact of phosphorylation on kinetic properties. In contrast to typical OmpR family members, the 'T/Y' switch orientation of SsoR resembles that of a phosphorylation-independent response regulator in *Helicobacter pylori*. Furthermore, as anticipated, the global iron homeostasis regulator Fur plays a key role in putrebactin synthesis regulation. Fur and SsoR function cooperatively to regulate the transcription of *pub* at multiple tiers, with Fur functioning as the sensor of iron starvation and SsoR as the major transcriptional activator. Subsequent investigations indicate Fur represses the transcription of *ssoR*. Overall, this study delineates the intricate, multi-tiered transcriptional, and post-transcriptional regulatory roles of key regulators in the siderophore synthesis of *S. oneidensis.*

B354

The use of an *in vitro* pig gut model to understand the influence of Cu supplementation on *Salmonella* Typhimurium

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Abstract

Reducing the spread of antimicrobial resistance (AMR) within the One Health compartments is one of the main challenges of controlling AMR. Historically, the feed of pigs has been supplemented with up to 170 mg/kg of copper (Cu), as an antimicrobial agent, which may have a detrimental influence on the gut microbiota. Additionally, Cu resistance has been associated with mobile genetic transfer of *Salmonella* Typhimurium ST34. However, the role of Cu supplementation and its influence on the gut microbiota have not been investigated. Thus, we employed our previously developed six-vessel continuous flow *in vitro* pig gut model to investigate the role of Cu resistance in *S.* Typhimurium and influence of supplementation on the pig gut microbiota. In addition, we developed an *in vitro* static pig gut model to facilitate multiplexing.

Using the *in vitro* pig gut model, we tested a wild type tagged strain (WITS) and various gene-deleted mutants of *S.* Typhimurium (Δsil, Δpco, ΔsopE and Δsil-pco-1) to identify the mechanisms of Cu resistance. The studies demonstrated that *S.* Typhimurium strains could replicate in the system. Moreover, supplementation with low Cu concentrations in the presence of faecal microbiota had a minimal influence on the CFU numbers of different *S.* Typhimurium WT and mutants, in comparison to no Cu added. However, the addition of high Cu concentrations resulted in a significant reduction in *S.* Typhimurium Δsil and Δsil-pco-1 bacterial concentrations. Furthermore, Cu supplementation resulted in an increase in other culturable faecal microbiota species. In conclusion, *Δsil* and/or *Δsil-pco-1* may have a role in Cu resistance.

B355

Characterisation of the *Neisseria cinerea* Type VI Secretion System Nucleases

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Abstract

The type VI secretion system (T6SS) is a specialised nanomachine prevalent across Gram-negative bacteria, implicated in inter-bacterial and bacteria-host interactions. The T6SS delivers toxic effectors to neighbouring cells through contact-dependent injection and contributes to shaping the microbiome through bacterial competition. Previous work has identified a plasmid-encoded T6SS in *Neisseria cinerea* CCUG 346T, which colonises the human upper respiratory tract. This is the first experimentally characterised T6SS in *Neisseria* spp. and is active against related pathogens *Neisseria meningitidis* and *Neisseria gonorrhoeae.*

The *N. cinerea* 346T plasmid encodes nine putative effector/immunity modules, five of which are predicted nucleases. Bioinformatic identification of conserved domains and sequence/structure homolog analysis reveals these are cargo effectors of the HNH and GIY/YIG nuclease families. Domain organisation and predicted structure of the *Neisseria* T6SS effector 3 (Nte3) is consistent with described secreted effectors in other species. Expression of the Nte3 C-terminal domain (Nte3-CTD) in *Escherichia coli* reduced cell growth and bacterial numbers, with toxicity being rescued in the presence of the cognate immunity protein. *In silico* HNH active site predictions identified key catalytic residues for mutation, which abrogated toxicity upon expression in *E. coli*. To confirm nuclease activity further, the degradation of genomic DNA was monitored following expression in *E. coli,* with clear degradation observed upon expression of Nte3-CTD.

Together, this data provides evidence for Nte3 as a T6SS nuclease. Preliminary secretome analysis suggests Nte3 is secreted upon T6SS-mediated attack, therefore playing an important role in antagonistic interactions within the microbiome, especially between commensal and pathogenic *Neisseria* spp..

B356

The role of response regulator, MtrD, in *Streptomyces* development and secondary metabolism

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Abstract

Streptomyces are gram-positive, filamentous bacteria integral in producing 55% of current clinical antibiotics. Usually, their lifecycle starts as a spore, germinates into mycelium, and then generates aerial hyphae which then sporulate for the cycle to begin again. *Streptomyces* harbour untapped potential as approximately 90% of their antimicrobials are not expressed under laboratory conditions. Further understanding of the regulatory pathways orchestrating antibiotic expression is required to ‘unlock’ these cryptic antimicrobials to address the escalating antimicrobial resistance crisis.

We investigate MtrD, a highly conserved regulatory protein that proves to be an important regulator for both lifecycle progression and possibly antimicrobial expression. The deletion of *mtrD* causes a large delay in *Streptomyces* lifecycle, which can be explained by the binding of MtrD to *wblA*, a gene required for the generation of aerial hyphae and sometimes linked with secondary metabolism. This finding shines further understanding into the development cycle of *Streptomyces.*

B357

The role of ColV plasmids in the fitness of avian pathogenic E. coli within the chicken gut microbiota

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Abstract

Extra-intestinal Pathogenic *Escherichia coli* (ExPEC) is a major pathogen of vertebrate hosts globally. Of particular concern is Avian Pathogenic *E. coli* (APEC), the leading cause of bacterial extra-intestinal disease in poultry, causing high morbidity, mortality, and economic losses. This diverse pathotype encompasses multiple genotypes from across the *E. coli* phylogeny; however, plasmids belonging to the ColV family represent a commonality between these distinct lineages. ColV plasmids, are known to promote survival and pathogenicity at extra-intestinal sites, however their impact on bacterial fitness within the intestinal reservoir remains unknown. We aimed to elucidate the contribution of APEC plasmids to bacterial fitness competition dynamics, carbon metabolism, and maintenance and proliferation within the microbial community in an *in vitro* avian gut model.

We identified a single, 155kb, conjugative ColV/IncF plasmid, designated pCB001, encoding classic APEC virulence genes and an integron conferring multi-drug resistance (MDR). Conjugation assays demonstrated pCB001 has the potential to transfer virulence genes and MDR to phylogenetically distant *E. coli* lineages. Generation of a pCB001-free host allowed direct comparison of plasmid-associated effects, whilst displaying no discernible effects on bacterial growth. Furthermore, use of an *in vitro* avian gut model allowed investigation on the impact of the plasmid on APEC maintenance and proliferation within the gut. Future work will focus on the transmission dynamics of APEC plasmids in the gut microbiota, to inform mitigation strategies, such as plasmid-targeted antigenic vaccines, to supress APEC carriage.

B358

Proteomic Analysis of *E. faecium* NCTC13169 in the Presence and Absence of Linezolid.

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Abstract

Background

Enterococcus faecium is a gram-positive bacteria found in the gastrointestinal tract of humans that can cause diseases such as endocarditis and meningitis. E. faecium has emerged as a worldwide nosocomial pathogen acquiring resistances to antibiotics such as linezolid. New methods of treating antibiotic resistant infections must be developed to combat this threat, otherwise it is predicted that by 2050 global deaths related to antibiotic resistant infections could rise to 10 million annually.

Methods

E. faecium NCTC13169 (WT) and a linezolid resistant NCTC13169 strain (cfrA) are grown with and without linezolid. Following incubation, the proteome of the samples are extracted. This extract is prepped for mass spectrometry via trypsin digestion and c18 resin filter tips.

Mass spectrometry data is processed using MaxQuant and then Perseus, which filters out any statistically insignificant results. Proteins in the samples treated with and without linezolid are compared and the fold change difference between samples are calculated.

Results/Discussion

Using this method, proteins with the greatest protein fold change in samples with linezolid versus those without are highlighted. In NCTC13169 WT, several proteins with the greatest fold changes between treatment and non-treatment groups appear to be stress related (ABC transporter ATP-binding protein/permease, YlbF family regulator). One protein with a fold decrease in samples with linezolid versus without is 23S rRNA (guanosine(2251)-2-O)-methyltransferase RlmB, which is linked to the linezolid inhibition mechanism. Mass spec results for linezolid resistant NCTC13169 are pending.

Conclusion

Proteomics is a valuable tool to understand both the mechanism of action and resistance of antibiotics.

B359

Bacterial heterogeneity produced by regulation and control of CRISPR-Cas Type I-F immunity in Pseudomonas aeruginosa

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Abstract

Bacteria need to protect themselves from infection and killing by bacterial viruses (phages), which outnumber them in larger quantities in diverse environments. For these reasons, bacteria have developed a sophisticated arsenal of **defence mechanisms** that can protect individual cells or the overall bacterial population. Individual protection is achieved via systems such as **CRISPR-Cas**, that are adaptive and of great interest for a wealth of biotechnological applications. Despite the large interest, their regulation and control under external signals and extracellular factors is poorly understood. Using ***Pseudomonas aeruginosa*** and the endogenous **Type I-F system**s our model study, we have engineered a series of **fluorescence-based reporter plasmids to track and identify different factors that are regulating the CRISPR-Cas loci.** Our data shows that this locus is **governed by different promoter regions,** regulating different aspects of the system, resulting in a heterogenous expression. We observed that growth rate directly modulates expression and that a subpopulation of cells expresses the CRISPR-Cas system at different growth stages maintaining a **constant level of immunity**. Furthermore, our results show that **viral infection can stimulate** the CRISPR-Cas promoters under our experiment conditions, suggesting that not only quorum-sensing signals could be regulating the system. We believe that such transcriptional heterogeneity can afford cells with bet-hedging opportunities that enable them to regulate the system, allowing them to adapt and survive in changing and stressful environments.

B360

Novel antibiotic potentiators to treat chronic Pseudomonas aeruginosa infections in the cystic fibrosis lung

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Abstract

Antibiotic resistance is a silent pandemic, responsible for millions of deaths each year. Resistance to treatments is especially problematic for vulnerable individuals with increased reliance on antibiotics, such as people with cystic fibrosis. Novel treatment options are urgently needed to address antimicrobial resistance on a global scale. One financially attractive option is to develop potentiators, which can extend the shelf-life of currently licensed antibiotics.

We are aiming to synthesise and develop novel antibiotic potentiators, with a focus on chronic Pseudomonas aeruginosa infections in the cystic fibrosis lung. These potentiators aim to both reduce patient treatment burdens and act as antibiotic resistance breakers.

Synthetic compounds which have been screened in silico and in vivo have been selected for their potentiating activity when combined with clinically relevant antibiotics. Bacterial strains used are clinical P. aeruginosa isolates from cystic fibrosis patients. Analogues of the selected compounds have been synthesised to try to increase their potentiating effectiveness. The analogues have been ranked according to potentiating efficacy, and to quantify their differences in activity.

When in combination, we find that some analogues act as antibiotic potentiators at various concentrations in vitro. They are successful at reducing the concentration of antibiotic required to achieve the reduced bacterial growth associated with minimum inhibitory concentration without potentiator. This highlights the potential for these compounds to act as resistance breakers in clinical P. aeruginosa isolates. The next steps will be to determine mode of action via membrane permeability assays and fluorescence and confocal microscopy. I have found that the primary compound is fluorescent, so we are currently investigating how this impacts stability, and whether the derivatives are also fluorescent.

B361

Effect of Fluoride on Growth Dynamics of Gut Microbial Strains

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Abstract

Topical fluoride can prevent dental caries by affecting the physiology of oral microbiota and inhibiting cellular enzymes. However, the effect of systemic fluoride on gut microbiota is unknown. This study investigates the impact of fluoride on the growth and viability of probiotic and non-probiotic strains, selected based on their importance in the human gut.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and growth dynamics were assessed on selected probiotics (*Lactobacillus fermentum, L. sakie, L. plantarum, L. buchneri, L. brevis, L. rhamnosus, L. paracasie, Bifidobacterium longum and B. breve*) and non-probiotic strains (*Escherichia coli*) under different fluoride concentrations (0.45 - 4500 mg/l)and bacterial growth (OD values) of control and test cultures was measured at 600nm absorbance in the spectrophotometer for every one-hour interval up to 24 hours. To estimate the *in vitro* pharmacokinetics of fluoride, fluoride concentrations in the medium in the presence and absence of bacteria were determined after 0, 48 and 96 hours by fluoride-ion-selective Electrode.

Probiotic strains were susceptible to fluoride at higher concentrations, where the chronic lethal concentration was 2250 mg/l. *E. coli* was found to be more tolerant to fluoride than probiotic strains. Growth densities of all probiotic organisms ($P \leq 0.01$) and *E. coli* ($P \leq 0.05$) were reduced on chronic systemic exposure. Fluoride concentration declined significantly ($p \leq 0.0001$) where the growth density of microorganisms was high, and non-significantly where growth density was low.

This study enhances our understanding of the impact of systemic fluoride on gut microbes, opening avenues for exploring the “fluoride-gut microbiome interaction.”

B362

Interaction of the polymyxin B binding to LPS-containing membranes from polymyxin-resistant Escherichia coli strains

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Abstract

The global crisis of multidrug bacterial resistance is continuously exacerbated by the persistent development of bacterial resistance to antibiotics. Polymyxin B, a positively charged peptide with a broad range of effectiveness against Gram-negative bacteria, is being reintroduced for utilization as a final option and as a supplementary treatment. To understand how polymyxin affects the bacterial cell membrane and morphology, a carboxyfluorescein (CF) release assay and scanning electron microscope were used to investigate the role LPS plays as a receptor for polymyxin. We confirm the efficacy of polymyxin against *E. coli* strains through agar diffusion assay and the minimal inhibitory concentration (MIC). The clinical isolates and the mcr-1 overexpressing *E. coli* strain exhibited a resistance ability by 4- to 16-fold in MIC toward polymyxin as compared to wild-type BW25113. LPS from the polymyxin-resistant strains showed a different CF release, suggesting LPS modification intervened in the release of CF. The analysis of cell morphology by SEM after polymyxin treatment caused a different level of roughness on the cell surface in a dose-dependent manner in wild-type *E. coli*, and the cell length was reduced from 2.2mm to 1.9mm when incubated with 32 times MIC of polymyxin. We observed that polymyxin will induce the change of cell morphology and rLPS with modifications will affect molecular recognition by polymyxin B.

B363

Metabolic reprogramming and altered cell envelope characteristics in a pentose phosphate pathway mutant increases MRSA resistance to β-lactam antibiotics

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Abstract

Central metabolic pathways control virulence and antibiotic resistance, and constitute potential targets for antibacterial drugs. In *Staphylococcus aureus* the role of the pentose phosphate pathway (PPP) remains largely unexplored. Mutation of the 6-phosphogluconolactonase gene *pgl*, which encodes the only non-essential enzyme in the oxidative phase of the PPP, significantly increased MRSA resistance to β-lactam antibiotics, particularly in chemically defined media with physiologically-relevant concentrations of glucose, and reduced oxacillin (OX)-induced lysis. Expression of the methicillin-resistance penicillin binding protein 2a and peptidoglycan architecture were unaffected. Carbon tracing and metabolomics revealed extensive metabolic reprogramming in the *pgl* mutant including increased flux to glycolysis, the TCA cycle, and several cell envelope precursors, which was consistent with increased β-lactam resistance. Morphologically, *pgl* mutant cells were smaller than wild-type with a thicker cell wall and ruffled surface when grown in OX. The *pgl* mutation reduced resistance to Congo Red, sulfamethoxazole and oxidative stress, and increased resistance to targocil, fosfomycin and vancomycin. Levels of lipoteichoic acids (LTAs) were significantly reduced in *pgl*, which may limit cell lysis, while the surface charge of *pgl* cells was significantly more positive. A *vraG* mutation in *pgl* reversed the increased OX resistance phenotype, and partially restored wild-type surface charge, but not LTA levels. Mutations in *vraF* or *graRS* from the VraFG/GraRS complex that regulates DltABCD-mediated d-alanylation of teichoic acids (which in turn controls β-lactam resistance and surface charge), also restored wild-type OX susceptibility. Collectively these data show that reduced levels of LTAs and OX-induced lysis combined with a VraFG/GraRS-dependent increase in cell surface positive charge are accompanied by significantly increased OX resistance in an MRSA *pgl* mutant.

B364

The kinase PknB modulates the cell wall biosynthetic machinery in *Streptomyces*

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Abstract

Streptomyces are filamentous bacteria best known for their ability to produce specialised metabolites (e.g. antibiotics). They grow as branching hyphal filaments to form a complicated mycelium. New growth zones are created by the formation of lateral branches. Proteins responsible for this process are organised in complexes called tip organising centres (TIPOCs). We have developed a screen to identify proteins suspected to interact (in)directly with DivIVA – member of TIPOC. Amongst other hits was PknB (Ser/Thr kinase), which is an essential protein in *Mycobacteria* and is involved in maintaining the cell shape. The exact role of PknB in *Streptomyces* hasn't been fully elucidated yet.

We have confirmed that deleting *pknB* rescues *Streptomyces* from the effects of overexpressing DivIVA. This prompted a comprehensive study into the impact of *pknB* deletion in *Streptomyces.* A mass spectrometry phosphoproteome analysis indicated that presence of PknB has effects on the phosphorylation state of CslA, a transglycosylase involved in the accumulation of β-glucans at mycelial tips. These β-glucan fibres were hypothesized to function as a 'bandage' around hyphal tips, which are susceptible to integrity loss due to continual cell wall remodelling.

Our research demonstrated that phosphorylation of CslA impacts its activity in β-glucan synthesis and influences the transcription of *divIVA*. This highlights one pathway through which PknB affects the stability of the cell wall structure.

Another mechanism involves sensitivity to antibiotics: the absence of *pknB* leads to heightened resistance to moenomycin, an antibiotic targeting transglycosylases. Importantly, the lack of RodA phosphorylation is integral to this increased resistance.

B365

Antibiotics and antibiotic resistance genes of the leafcutter ant microbiome

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Abstract

Leafcutter ants are expert farmers whose colonies obligately depend on the maintenance of a fungal garden. Freshly-cut leaf material is provided to the cultivar which concentrates digestible nutrients into structures known as gongylidia. *Escovopsis* is a historic member of the colony microbiome with a largely parasitic role. If not addressed, *Escovopsis* outcompetes the cultivar and degrades its hyphal structures through several virulence mechanisms. Countering this, leafcutter ants have developed a protective microbiome dominated by the horizontally-transmitted, antifungal-producing *Pseudonocardia* bacteria. The effects of this are two-fold; a chemical defence against the *Escovopsis* mycoparasite, and a selective pressure within the ant cuticular microbiome, ensuring only beneficiaries persist. *Streptomyces* and other members of the Actinobacteria family are known to be vertically acquired into the ant cuticular microbiome, likely due to the presence of a number of antibiotic resistance genes. This ecosystem is therefore an intriguing model in which to study how antibiotics and antibiotic resistance genes shape a protective microbiome. Additionally, the range of antibiotic compounds produced within the cuticular microbiome by mutualists remain largely unknown. *Streptomyces* in particular are predicted to produce a number of antibiotic compounds encoded for on biosynthetic gene clusters that are 'silent' under laboratory conditions. The leafcutter ant cuticular microbiome may therefore also serve as a platform on which to discover novel natural products, forming a response to growing antimicrobial resistance and a wane in natural product discovery. The aim of my project is to identify the antibiotics and antibiotic resistance genes that shape the leafcutter ant microbiome.

B366

Characterisation of metal-homeostasis systems in the human gastric pathogen Helicobacter pylori

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Abstract

Transition metals are essential cofactors for approximately a half of all enzymes, a third of all proteins, yet are toxic in excess. These properties are exploited by host innate immune defenses that manipulate metal levels to attack invading microbes by metal-deprivation and/or exposure to metal-excess. In response, microbes must employ various strategies to acquire sufficient of each metal to meet the metal demands of their proteins whilst avoiding metal-poisoning. These metal homeostasis systems often represent key bacterial virulence factors. The human gastric pathogen *Helicobacter pylori* infects roughly half of the world's population and is a major risk factor for gastric ulcers, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma. Whilst maintaining metal homeostasis is crucial for gastric colonization by *H. pylori* and metals are exploited in current treatment regimes, the mechanisms employed by *H. pylori* to sense and respond to changing metal levels within its host remain largely uncharacterized. Our research is focused on the identification and characterization of such systems, including revisiting the functions of the previously reported P-type ATPase CadA and Czc-type resistance-nodulation-cell division (RND)-type exporter CznABC. We confirm CadA as the primary zinc export system of *H. pylori*, with Δ*cadA* mutant being extremely sensitive to zinc and over-accumulate zinc, whilst CznABC provides an additional level of defense against zinc toxicity. We also reveal new insights regarding the function of CznABC with respect to metal resistance. Our work regarding the characterization of these metal handling systems in *H. pylori* will be described.

B367

Evaluating novel antimicrobials against MRSA by targeting pathways leading to the essential signalling nucleotide c-di-AMP causing altered susceptibility to beta-lactam antibiotics.

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Abstract

Maintaining the effectiveness of existing antimicrobial drugs or finding ways to reintroduce drugs to which resistance is widespread is an important part of efforts to address the challenge of antimicrobial resistance. Predominantly the safest and most effective class of antibiotics are the b-lactams, which are no longer effective against methicillin resistant *Staphylococcus aureus* (MRSA). We report that the purine nucleosides guanosine and xanthosine have potent activity as adjuvants that can resensitise MRSA to oxacillin and other b-lactam antibiotics. Mechanistically, exposure of MRSA to these nucleosides significantly reduced the levels of the cyclic dinucleotide c-di-AMP, which is required for b-lactam resistance. The activity of nucleoside/beta-lactam combinations can be further enhanced by including other drugs that also interfere with staphyloccocal metabolism. Drugs derived from nucleotides may have clinical potential to restore or enhance the therapeutic effectiveness of b-lactams against MRSA and potentially other AMR pathogens.

B368

Engineered *Lactococcus lactis* for lycopene production

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Abstract

Retinoids and their precursors constitute a family of molecules, including lycopene, β-carotene, retinal, retinol (vitamin A), and retinoic acid. Widely used in biomedicine, as well as in the food and cosmetic industries, these compounds are valued for their antioxidant, anti-inflammatory, and antiaging properties. Traditionally extracted from fruits and vegetables, the conventional methods involve lengthy and costly procedures, often leading to degradation or oxidation.

In this context, *Lactococcus lactis* emerges as a promising food-grade expression platform to produce proteins and bioactive compounds, such as lycopene, in situ. Moreover, the genetic expression can be controlled by the NICE system, which uses nisin -a food additive with a 50-year history- to induce such synthesis with a linear response.

To synthesize lycopene, the engineered metabolic pathway must incorporate phytoene synthase (*crtB*) and phytoene desaturase (*crtI*), converting geranyl-geranyl pyrophosphate (GGPP) into phytoene and subsequently lycopene. Typically, GGPP synthase (*crtE*) is engineered to increase the concentration of GGPP, a crucial precursor in the pathway. Despite limited research utilizing *Lactococcus lactis* for lycopene production, some studies have engineered DNA sequences coding for these enzymes from diverse organisms, such as *Deinococcus radiodurans* [1] and *Pantoea ananas* [2], with promising results in gut protection against oxidative stress.

This study introduces an alternative strategy for lycopene production, employing the pTRKH2 shuttle vector, different *L. lactis* strains (NZ9000 and NZ9020), varied culture conditions, and culture media.

References:

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B370

The oxidative stress response of Aspergillus fumigatus highly depends on glucose and iron availability

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Abstract

Fungal pathogens often cope with stresses, including oxidative, iron- and carbon-limitation stress in the human body. To deeper understand how combined iron-carbon limitation alters the oxidative stress response, *Aspergillus fumigatus* was cultured on glucose-peptone or peptone-containing media with or without deferiprone (DFP) added as an iron chelator. After adaptation to the conditions, cultures were treated with H_2O_2 and transcriptome changes were recorded by RNA sequencing. Focusing on iron homeostasis genes, we found that carbon limitation alone had little effect. Only heme-binding protein genes were enriched in both the upregulated and the downregulated gene sets. Iron limitation altered iron metabolism in a very similar manner regardless of the presence of glucose: Iron acquisition genes were enriched in the upregulated, whereas Fe-S cluster protein and heme-binding protein genes were enriched in the downregulated gene sets. Interestingly, the oxidative stress-induced transcriptional changes in iron metabolism genes were highly dependent on the availability of glucose and iron: H_2O_2 treatment upregulated Fe-S cluster assembly genes only in iron-limited cultures, whereas catalase and peroxidase genes showed upregulation only in iron-limited peptone cultures. Fe-S cluster protein genes were enriched in the upregulated, whereas the heme-binding protein genes were enriched in the downregulated gene set in glucose-peptone and iron-limited peptone cultures. Iron acquisition genes were enriched in the upregulated gene set on glucose-peptone and in the downregulated gene set on peptone. Such flexibility of the oxidative stress response may be crucial for efficient adaptation to adverse environmental conditions that pathogens must cope within the human host.

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B371

Profiling the metabolomic differences between methicillin-resistant *Staphylococcus aureus* isolates derived from invasive and non-invasive clinical infections

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Abstract

Introduction: Deciphering the metabolic distinctions between methicillin-resistant *Staphylococcus aureus*(MRSA) isolates associated with different clinical manifestations could yield insight into virulence mechanisms and identify potential biomarkers. This study aimed to compare the metabolic profiles of MRSA isolates associated with diverse clinical presentations.

Methods: MRSA obtained from blood cultures (n=23) were categorized as invasive isolates. Those obtained from superficial skin infections (n=49) and nasal colonizers (n=24) from screening anterior nares swabs were categorized as non-invasive. Intracellular metabolites from bacterial colonies on blood agar were extracted using the methanol extraction method. Extracted metabolites were analyzed using a TimsTOF mass spectrometer with an Apollo II electrospray ionization source. Metabolomic data analysis was done on MetaboScape® 4.0.

Results: A total of 150 metabolites were identified across all isolates. Isolates from superficial skin infections and nasal colonizers did not show significant differences in metabolite intensities. Compared to the non-invasive isolates, the invasive isolates had two metabolites (Sphinganine and Phosphoserine) which were consistently recovered at higher intensity. Additionally, there were three metabolites (Cytidine, Benzoic acid, and Guanosine) with significantly lower intensity in the invasive isolates. Over-representation analysis of enriched KEGG pathways for these five differentially abundant metabolites revealed the Sphingolipid metabolism pathway to be the most significantly enriched ($p<0.05$).

Conclusion: Our findings indicate metabolic targets unique to invasive MRSA isolates. The enrichment of a pathway which is well-linked with pathogen invasiveness provides insight into the virulence mechanism of these isolates. Further work to investigate these targets as potential biomarkers is warranted.

B372

Role of 2,7-anhydro-Neu5Ac in the crosstalk between commensal and pathogenic bacteria in the gut.

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Abstract

Sialic acids are an abundant nutrient source for mucosa-associated gut microbes. While Neu5Ac is the main form of sialic acid released by bacterial species in the gut, the human gut symbiont *Ruminococcus gnavus* releases 2,7-anhydro-Neu5Ac, through the action of an intramolecular *trans*-sialidase (IT-sialidase). We previously showed that *R. gnavus* expresses a 2,7-anhydro-Neu5Ac-specific transporter and an oxidoreductase catalysing the reversible conversion of 2,7-anhydro-Neu5Ac to Neu5Ac that are essential for the capacity of *R. gnavus* ATCC 29149 to utilise 2,7-anhydro-Neu5Ac. This unique sialic acid metabolism pathway provides *R. gnavus* with a competitive advantage in the gut for colonisation of the mucus niche.

Bioinformatics analyses revealed the presence of oxidoreductase homologues across Gram-negative and Gram-positive bacterial species and co-occurrence with sialic acid transporters, suggesting possible cross-feeding of 2,7-anhydro-Neu5Ac released by *R. gnavus*. We showed that *E. coli* BW25113 harbours a sialic acid transporter (YjhB) and an oxidoreductase (YjhC) which are both crucial for 2,7-anhydro-Neu5Ac metabolism. Additionally, we identified homologs of these genes in Salmonella SL1344 and showed that this strain could utilise both Neu5Ac and 2,7-anhydro-Neu5Ac *in vitro*, albeit to different extent, while a Salmonella mutant lacking these genes lost the capacity to grow on 2,7-anhydro-Neu5Ac, which was restored following complementation with *E. coli* YjhB and YjhC. Preliminary mouse experiments showed that *R. gnavus* ATCC 29149 has a protective effect on Salmonella infection as compared to a *R. gnavus* Nan mutant which lacks the ability to release and metabolise 2,7-anhydro-Neu5Ac.

B374

A new twist on drug design: modifying metabolism to inhibit virulence

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Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) is a human pathogen that causes bloody diarrhoea, haemorrhagic colitis, and life-threatening haemolytic uremic syndrome. We are focused on the development of alternative strategies to the use of antibiotics to treat EHEC infections. A family of anti-virulence compounds, the salicylidene acylhydrazides (SA), cause an attenuation of virulence and a reduction in expression of the Type Three Secretion System (T3SS), the major system used by EHEC to attach to host cells. One target of these compounds is a bacterial bidirectional enzyme called AdhE that catalyses the conversion of acetyl-CoA to ethanol. Deletion of the *adhE* gene in EHEC causes the same similarities in phenotypes as seen when the SA compounds are added to this strain. Unusually, AdhE oligomerises *in vivo* and *in vitro* to form filaments heterogenous in length called spirosomes.

Techniques including analytical ultracentrifugation (AUC) sedimentation velocity analysis, enzymatic assays and super-resolution single-molecule and FRET microscopy were performed to understand why and how AdhE spirosomes are formed and how this process is regulated. The results suggest that AdhE spirosome formation is necessary to balance the two directions of the enzymatic reaction. AdhE was incubated with one of the SA compounds called ME0054, to elucidate its effect on AdhE spirosomes. Techniques previously mentioned in addition to transmission electron microscopy (TEM) showed that ME0054 disrupts the spirosomes, thereby enhancing the conversion of ethanol in acetyl-CoA. This effect could be related to a change in the levels of acetylation of proteins linked with EHEC virulence.

B375

The *Bacillus subtilis* S8-PTS: Investigating a novel polymorphic toxin system in Gram-positive bacteria

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Abstract

Bacillus subtilis is a widespread Gram-positive, non-pathogenic bacterium that can act as a plant-growth-promoting rhizobacterium (PGPR) via the formation of root-associated biofilms. For *B. subtilis'* use as a biofertilizer to be fully realised, a beneficial strain must outcompete *B. subtilis* isolates that already reside on a plant's root. This necessitates elucidating the molecular mechanisms that facilitate one *B. subtilis* strain's dominance over another.

Here we investigate a polymorphic toxin system (PTS) recently uncovered within Gram-positive bacterial species: the S8-peptidase-associated polymorphic toxin system (S8-PTS). Using our library of sequenced *B. subtilis* environmental isolates, we identified multiple strains that possess S8-PTS gene clusters, enabling us to investigate this system's ability to mediate inter-bacterial competition in *B. subtilis.*

We first employed *E. coli* as a heterologous protein expression host, confirming the existence of a toxin-immunity gene pair associated with a *B. subtilis* S8-PTS. When overexpressed within *E. coli*, an S8-PTS toxin produced marked growth inhibition while co-expressing the predicted immunity protein protected against this toxin's anti-bacterial activities. Furthermore, by utilising an engineered *B. subtilis* strain possessing an inducible S8-PTS within a fluorescence-reporter-based competition assay we confirmed this system's ability to mediate inter-bacterial antagonism against a vulnerable *B. subtilis* competitor.

The S8-PTS likely represents a significant inter-bacterial weapon employed by both Gram-positive PGPR bacterial species (e.g., *B. subtilis)* and pathogens (e.g., *Staphylococcus* aureus and *Listeria monocytogenes).* An understanding of how this PTS functions will enhance our understanding of *B. subtilis* intraspecies competition mechanisms and those weapons deployed by Gram-positive bacterial species more generally.

B377

Genome-scale metabolic modelling trimethoprim's mode of action against Uropathogenic *Escherichia coli* for rapid point-of-care susceptibility testing

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Abstract

There is a long history behind ATP bioluminescence as a cost-effective and rapid means of detecting bacteria and antibiotic susceptibility for clinical use. Treatments for common illnesses like urinary tract infections (UTIs) rely on empirical or delayed antibiotic treatments and would benefit greatly from technology like ATP bioluminescence. However, the challenge has been achieving rapid susceptibility results for all clinically used antibiotics like trimethoprim. The aim was to understand the causal mechanism of ATP depletion during trimethoprim challenge and devise a way of detecting trimethoprim susceptibility using ATP bioluminescence within 30 minutes. Flux balance analysis (FBA) was performed on a genome-scale metabolic model of *E. coli* UTI89 incorporating *in vivo* ATP data to identify the role of various media components and ATP during trimethoprim challenge. Modelling predictions were supported by screening 96 clinical Uropathogenic *E. coli* isolates in different media conditions. Purines were identified as a prohibitive factor in rapid trimethoprim susceptibility testing. By excluding purines, trimethoprim susceptibility results could be achieved in 30 minutes of exposure. FBA suggested free ATP was salvaged to restore trimethoprim-induced purine and nucleoside depletion needed for DNA and RNA synthesis. This work provides novel insights into how bacterial metabolism can be manipulated to achieve rapid trimethoprim susceptibility testing with potential applications in point-of-care testing for UTIs.

B378

Salmonella’s susceptibility to azithromycin can be detected in a matter of hours with nanomotion-based technology

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Abstract

Introduction | Azithromycin is a bacteriostatic macrolide antibiotic used for treating invasive salmonellosis. Interestingly, azithromycin's minimal inhibitory concentration (MIC) is significantly higher than concentrations achievable in serum. The aim of the work was to find out if the sensitivity to the bacteriostatic antibiotic can be determined in real time and faster than with traditional MIC testing and to associate the sensitivity with molecular mechanisms.

Methods | Traditional antimicrobial susceptibility testing (AST) was coupled with the Resistell AST technology measuring the changes in nanomotion caused by physiologically active bacterial cells in response to the antibiotic. Efflux-efficient mutant and acidic pH altering susceptibility to azithromycin were tested in addition to standard Salmonella strain at neutral pH. Additionally, a fluorescent bioreporter was exploited to detect changes in translational activity in response to the drug and ethidium bromide (EtBr) assay was used to estimate membrane permeability and efflux.

Results | Nanomotion-based AST revealed that it is possible to determine susceptibility of Salmonella to azithromycin within two hours. The increase of the variance of the nanomotion signal peaked just below MIC, implying activation of stress responses to maintain tolerable intracellular drug concentrations. Bioreporter assays showed that at acidic pH intracellular azithromycin concentration was significantly lower than extracellular. EtBr assay showed less accumulation at subinhibitory azithromycin concentrations, indicating upregulated efflux.

Conclusion | AST based on nanomotion detection is suitable for detecting Salmonella susceptibility to azithromycin. Detection can be achieved within a couple of hours. Results suggest that Salmonella can build up azithromycin concentration gradient affected by drug permeability and efflux.

B380

The role of the human urine and host-microenvironment on treatment-failure against uropathogenic *E. coli* (UPEC)

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Abstract

Antimicrobial resistance (AMR) has been rapidly spreading worldwide and has been identified as one of the most pressing issues in modern medicine by the World Health Organisation (WHO). Urinary tract infection (UTI) is prevalent worldwide and could be considered a major driver of AMR due to the high rates of re-infection and elevated treatment failure.

The gold standard methodology for antimicrobial testing is determined using minimum inhibitory concentration (MIC) against bacteria. However, even if the techniques allow standardisation and reproducibility, they fail to recapitulate relevant physiological conditions associated with host pathogen interaction and microenvironment.

We have developed a state-of-the-art 3D urine-tolerant human urothelium (3D-UHU) organoid model. The urine microenvironment influences bacterial behaviour and, in combination with the host-pathogen interactions, might significantly affect treatment response. Clinical uropathogenic E. coli (UPEC) isolates were selected and differential gene expression was compared in standard media, human urine and in the 3D-UHU. Following this antimicrobial efficacy of panel of commonly used antibiotics was investigated in a human urine-containing media and a standard media against a UPEC isolates.

Our finding supports that the urine microenvironment and host interaction are key in the behaviour of UPEC. The antimicrobial response of UPEC in standard media might not be representative of a UTI infection, and factors such as human urine or a bladder organoid might be used to reduce the failure of drugs and in the pipeline for new drug discovery

B381

Taxonomic classification of rumen bacteria by MALDI-TOF Mass Spectrometry - Custom database development

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Abstract

The rumen microbiome consists of a diverse ecosystem of microbes, mainly anaerobic bacteria, protozoa, fungi, and archaea, which enable ruminants to make energy stored in plant material metabolically available. The understanding of the rumen microbiome remains poor due to the complexity of culturing anaerobic microbes and the high requirements in cost, time, and skilled labour to identify them with traditional methods. In this study we assessed the capability of MALDI-TOF Mass Spectrometry (MS) to identify ruminal bacteria compared with 16s rRNA sequencing and established a custom and specific database for rumen study. 150 isolates were obtained from bovine and ovine ruminal fluid samples using the dilution-to-extinction method on three culturing media: Hobson M2, PC basal, and Brain Heart Infusion (BHI). The isolates were analysed by MALDI-TOF MS with the Extended sample pretreatment protocol. However, the initial analysis of 60 of the isolates with MALDI-TOF MS yielded an identification score of < 6.0 classifying the identification result as 'Unreliable' for all isolates tested. Currently, a custom ruminal MALDI-TOF MS database is being constructed, utilising the Extraction sample pre-treatment protocol and recent ruminal isolates previously identified by full 16S sequencing. The data-based validation has been conducted simultaneously using the Extended protocol, resulting in a significant enhancement of the reliability and accuracy of the identification results. In conclusion, this study illustrates that the current MALDI-TOF MS database is not sufficient to identify rumen microbes. However, with the creation of a custom database this high-throughput technology can have major benefits for identifying these critical microbes.

B382

Exploring *Treponema pallidum* Attachment and Morphology in the *in vitro* Co-Culture Systems: Implications for Syphilis Research

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Abstract

Syphilis, caused by *Treponema pallidum* subsp. *pallidum* (TPA), continues to pose significant global health challenges due to its rising incidence and impact on neurological, cardiovascular, and pregnancy health. Understanding the biology of TPA has been limited for many years due to the inability to culture this bacterium *in vitro*. By employing recently introduced co-culture methods with mammalian epithelial cells (Sf1Ep), we gain novel insights into TPA's attachment and morphology.

Culturing TPA in a 1.5% oxygen environment for seven days, we observed the majority of treponemes adhering to the Sf1Ep monolayer, with about 10% remaining suspended. This distinction led us to separately culture the planktonic treponemes, revealing their eventual attachment to Sf1Ep cells and indicating dynamic interactions within the culture. To examine morphological differences, cells from both attached and planktonic fractions were analysed using Scanning Electron Microscopy (SEM). Our findings showed significant length variations between planktonic and attached fraction indicating the process of division. Furthermore, we observed various attachment patterns in TPA, which adhered tightly to Sf1Ep cells. And finally, the Sf1Ep cells showed no morphological alterations due to infection under SEM, although their division time increased when co-cultured with TP, suggesting a complex interplay.

Our study provides insights into TPA's behaviour *in vitro*, potentially illustrating a dynamic process of attachment, release, and reattachment in its interaction with Sf1Ep cells. These findings are crucial for understanding TPA's behaviour *in vivo* and have implications for *in vitro* culture optimisation and syphilis prophylaxis.

B383

Understanding the temporal dynamics of post-segregational killing in bacteria

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Abstract

Shigella sonnei is a leading contributor to the global burden of diarrheal disease and causes about 188 million cases of shigellosis annually. *Shigella* possess a 210 kb, single copy, non-conjugative virulence plasmid (pINV) which plays an indispensable role in the pathogenesis of infection. To maintain this crucial plasmid in the population, the plasmid encodes Toxin:Antitoxin (TA) systems, which increases its vertical transmission. If a plasmid encoding a TA system is lost, antitoxin and toxin synthesis terminates and the more stable toxin kills the plasmid–free cells, through a phenomenon called post-segregational killing (PSK). The VapBC TA system in *S. sonnei* pINV is necessary for plasmid maintenance and once pINV is lost, toxin VapC cleaves the initiator tRNA (tRNAfMet), to inhibit translation in plasmid-free cells.

The overall aim of this study was to unravel the process of PSK by detecting pINV loss at the single cell level and visualize the events that culminate in cell death. For this, we developed a fluorescent tag for tracing pINV and tracked the fates of individual cells after plasmid loss using time-lapse microscopy combined with mother-machine microfluidic chips. We found that PSK was mediated by VapC toxin and involved a series of changes in cell morphology, viability, translational rates, and growth arrest eventually leading to cell death.

B384

ADP-ribosylation of DNA regulates cellular function in *Mycobacterium tuberculosis*

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Abstract

The bacterial DarTG toxin-antitoxin system is present in a number of important pathogens including the *Mycobacterium tuberculosis* complex, enteropathogenic *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The DarT toxin is able to add ADP-ribose from nicotinamide adenine dinucleotide (NAD+) onto thymidine in a sequence-specific manner in ssDNA. Unregulated expression of DarT results in arrest of DNA replication, induction of the mutagenic SOS-response, and ultimately death of the bacterium. The DarG anti-toxin is able to both inhibit the activity of the DarT toxin and reverse the ADP-ribosylation of thymidine. Structural study of DarT from *Thermus sp*, reveals the highly coordinated interactions between DarT and its DNA target which enable the sequence specific addition of ADP-ribose to the thymidine base. We have demonstrated the sequence specificity of *M. tuberculosis* DarT to be the motif TTTW and we identified ADP-ribosylation of the AT-rich origin of replication (oriC) in mycobacteria. Together with an elevated growth rate of DarTG mutant *M. tuberculosis*, this suggests a fundamental physiological role for the DarTG system as a molecular switch coordinating replication. Here, we further examine the role of DarTG in replication and as an epigenetic regulator of gene expression.

B385

The impact of air pollution on Staphylococcus aureus

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Abstract

According to the world health organisation, air pollution from fine particular matter (PM) caused 4.2 million premature deaths worldwide in 2019. PM is released as a result of industrial and vehicle emissions, as well as brake and tyre wear. It has been shown that exposure to PM, of which black carbon (BC) is a major component, not only has harmful effects on human respiratory systems, but also alters the way the bacteria that colonise us behave. In our recent publications we have shown that *Staphylococcus aureus* has an increased ability to adhere to and invade respiratory epithelial cells after exposure to and co-exposure with BC, as well as *in vivo* an increased colonisation of the murine respiratory tract, and alterations to biofilm antibiotic tolerance. We have shown BC upregulates genes related to toxin production, immune evasion, and cellular transport, as well as downregulating genes relating to metabolism. Following on from this we are aiming to understand mechanisms underpinning the BC response. The genetic mechanisms behind these effects and the potential regulators are being explored along with BC-induced changes to epithelial cells and their infection response.

B386

Conductive grain dependent electron exchange in methanogenic Baltic Sea consortia oxidizing acetate

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Abstract

Acetate is a key intermediate in organic matter mineralization, particularly in the deep subsurface, with limited electron acceptors. Here, methanogenic archaea can split acetate to CH_4 and CO_2, while acetate-oxidizing bacteria can oxidize it to CO_2 providing reducing equivalents for methanogens.

Our work studies the effects of conductive particles on acetate metabolism in marine sediments. In Bothnian Bay sediment enrichments, granular-activated-carbon (GAC) and magnetite were crucial for linking syntrophic acetate oxidation (SAO) with CO_2-reductive methanogenesis, known as CIET – Conductive particle mediated Interspecies Electron Transfer.

Previous findings showed a fourfold increase in methanogenesis with conductive particles (unlike unamended controls). Labeled acetate was mainly assimilated by a Geobacterales-like organism, converting the $^{13}CH_3$-group on acetate to $^{13}CO_2$. Inhibition experiments using a methyl-CoM inhibitor or antibiotics confirmed metabolic co-dependency between Archaea and Bacteria.

Metagenomic analyses revealed distinct consortia compositions, leading to the identification of core CIET organisms exclusively in incubations with conductive particles. Metagenome-assembled genomes (MAGs) from these particles include two main players, a Geobacterales and a Methanosarcina only found in conductive particle incubations (other groups within the consortia may contribute to secondary functions).

The Methanosarcina, typical of marine environments, harbors an Rnf-operon flanked by a membrane-bound methanogenesis multiheme cytochrome capable of bidirectional Extracellular Electrons Transfer (EET). The *Geobacterales*-MAG showcases over 40 multiheme c-type cytochromes, acetate transporters, and a complete acetate-oxidation pathway, confirming their potential for acetate-oxidation, and EET-capabilities.

Our results highlight complex microbial interactions and diverse strategies in CIET, providing valuable insights into the metabolic capabilities of marine sediment consortia contributing to methane emissions.

B387

Repression of a potent genotoxin via amino acid supplementation

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Abstract

Many *Escherichia coli* strains of the B2 phylogroup synthesise colibactin, a potent genotoxin biosynthesised by the *pks* island. In eukaryotic cells, colibactin induces DNA damage, chromosomal instability, cell-cycle arrest, and is implicated in the development of colorectal cancer. Our results demonstrate the inhibitory effect of several amino acids on colibactin production in *pks*$^{+}$ strains, with particular repression seen with D-Serine. The ability of proteinogenic L-amino acids and corresponding D-enantiomers to repress colibactin production was measured via transcription of the *clbB* gene - necessary for the biosynthesis of the genotoxin - through a p*clbB*-GFP reporter-assay in various types of growth media. The most inhibitory amino acids were validated by RT-qPCR and were selected for analyses in prototypical and clinical colibactin-producing strains. D-Serine and D-Tyrosine emerged as the most repressive amino acids: in the presence of D-Serine, exposure of plasmid DNA to colibactin resulted in an 2.05-fold decrease in levels of cross-linked DNA, whereas in the presence of D-Tyrosine, exposure of plasmid DNA to colibactin resulted in an 3.43.-fold decrease in levels of cross-linked DNA. We observed that D-Serine furthermore reduces the cytopathic responses typically observed during infection of HeLa cells with *pks*$^{+}$ strains. The effectiveness of D-Serine *in vivo* is being studied in an ongoing experiment, involving C57BL/6 mice infected with the colibactin-producing strain EcN in an AOM/DSS background. While our *in vivo* results are yet to be determined, our *in vitro* ones show the potential of D-Serine as a preventative in colibactin-associated disease and implicate several other amino acids as possible therapeutic candidates.

B388

Protein antibiotic import mechanisms: understanding and engineering the novel bacteriocin KvarM

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Abstract

New strategies must be developed to combat infections by increasingly drug resistant bacteria. The common hospital-acquired Gram-negative pathogen *Klebsiella* is a high priority target, with alarming rates of antimicrobial resistance. Bacteriocins are antimicrobial proteins deployed by bacteria against closely related neighbouring cells, with potential to be developed into alternatives to traditional antibiotics.

KvarM is a 30.8 kDa bacteriocin recently identified from *Klebsiella varicola* through its homology with the peptidoglycan degrading *E. coli* bacteriocin Colicin M. It is particularly interesting as it was shown to be active against a wide spectrum of *Klebsiella* strains, including those that are multidrug resistant in plate, liquid, and biofilm killing assays indicating that it might be a good candidate for future development of a *Klebsiella*-specific antimicrobial. In order to understand KvarM translocation and the degree and mechanism of its species-specific killing, we are focusing on its interaction with its outer membrane receptor FhuA.

Thus far, we have characterised KvarM's target strain specificity, shown that its outer membrane receptor is FhuA, and that its translocation across target cell envelopes is energised by the Ton system. We have formed a complex between KvarM and FhuA *in vitro* with the aim of solving the structure of the complex using cryo-electron microscopy. This would be the first structure of an M type bacteriocin in complex with its outer membrane receptor.

Long term, we aim to harness our understanding of KvarM killing to develop novel uptake mechanisms for bacteriocins, designing synthetic bacteriocins with potential for development into precision antimicrobials.

B389

Genome conservation and differences between butyrate producing *Roseburia intestinalis* human gut isolates from different geographical locations

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Abstract

Roseburia intestinalis is one of the most abundant human gut bacteria that produces butyrate from a variety of dietary polysaccharide substrates and plays an important role in maintaining gut health. Individuals with inflammatory and other metabolic diseases frequently harbour lower levels of these bacteria, highlighting its importance in maintaining gut homeostasis. We have used whole genome sequence data from sixteen selected *R. intestinalis* strains isolated from healthy human gut, from different geographical locations, and performed pangenome analysis. We investigated its antimicrobial resistance potential and carbohydrate utilisation capabilities. The results demonstrated that *R. intestinalis* strains exhibited an open pan-genome structure. Phylogenetic analysis of the core genome showed regional clustering of the strains based on their origin of isolation (Asia, Europe and America) indicating geographical stratification. In total, 295 genes were involved in carbohydrate degradation and contained at least one CAZy domain. 96 of these domains were present in all sixteen strains, signifying considerable inter-strain conservation. Interestingly, the majority of *the R. intestinalis* strains (10/16) harboured the tetracycline resistance genes *tet*(O) or *tet*(40). The results showed considerable conservation between the *Roseburia intestinalis* genomes, whilst also revealing region-specific differences indicating that specific expansions have occurred in different habitats. The identification of tetracycline resistance genes in these strains warrants a careful evaluation of their suitability as probiotic and live-biotherapeutic candidates to minimize the risk of transferring resistance to other resident gut bacterial populations.

B390

Campylobacter pathogenesis: reactive oxygen species, mechanisms of bacterial disease and host defence modulation in the context of human disease

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Abstract

Infectious diarrhoea is a global problem with *Campylobacter* being the most common bacterial cause. Specifically, the species *Campylobacter jejuni* are associated with over 80% of Infectious diarrhoea cases. Symptoms typically including bloody diarrhoea, fever and abdominal pains. Despite its importance, the mechanisms by which Campylobacter infection promotes disease in humans remain unclear.

Reactive oxygen species (ROS) are a group of oxygen-based chemical intermediaries with an uneven number of electrons. ROS production is mediated by the activation of the nitrous oxide (NOX) pathway and has shown to have an antimicrobial role. *Campylobacter* can modulate ROS production pathway components to aid intracellular proliferation, downregulate NOX1 and antioxidant defence genes *CAT* and *SOD1*.

The project will investigate ROS interplay in *Campylobacter* mediated infection – before, during and after activation. Campylobacter mutants lacking certain virulence determinants will be tested for their ability to invade host cells and activate the NOX pathway. Transcriptional analysis of bacterial and host cells during invasion assays will elucidate genomic changes of both agents during the invasion cycle. The biochemical basis of signalling pathways will be investigated, using proteomic techniques. Then, the project will combine 'Omic' technologies to develop understanding of ROS reacting to *C. jejuni* infection in the context of inflammatory processes. Other aims of the project include developing a 3D cell culture model to better recapitulate *in vivo* environments.

The approaches used in this project will lead to a better understanding of *Campylobacter* interactions with host cells and will provide a platform from which to test basic host pathogen interactions for other species of bacteria and viruses.

B391

Molecular basis for emergence and success of an emergent lineage of *emm4* Group A Streptococcus

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Abstract

Group A Streptococcus (GAS) is a gram-positive bacterium and human pathobiont, that is the causative agent of a diverse array of infections including scarlet fever, necrotising fasciitis and rheumatic fever. A major driver of changes to GAS epidemiology and virulence is clonal replacement, a process by which new variants of an existing lineage emerge. A new clonal lineage of serotype M4 GAS has been reported in Europe and the US, and is associated with enhanced virulence, however the mechanisms underpinning this change remain poorly understood. We have identified distinct genetic changes associated with this emergent lineage; notably degradation of all associated prophage and a novel cellular fusion protein and sought to define their impact on bacterial fitness.

Growth curves on ancestral and emergent isolates treated with the prophage inducing agent mitomycin C were performed. This showed a significant reduction in prophage mediated bacterial cell lysis compared to the ancestral lineage. Furthermore, the susceptibility of each lineage to major classes of antibiotics was also quantified via broth microdilution assays. This indicated increased resistance to several antibiotics in the emergent lineage. Additionally, the presence of biofilms was quantified using biofilm assays. This also revealed an improved biofilm formation in the emergent lineage.

Overall, we demonstrate that the emergent lineage is associated with enhanced fitness through genetic changes that facilitate increased resistance to several classes of antibiotics, improved biofilm and a reduction in prophage mediated bacterial lysis. Our data indicates a concerning trend in the evolution of GAS towards enhanced persistence and AMR.

B392

c-di-GMP regulates meropenem susceptibility in concert with the sRNA ErsA in *Pseudomonas aeruginosa*.

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Abstract

In *Pseudomonas aeruginosa*, meropenem resistance is influenced by the expression of the OpdP and OprD porins. These porins are used for the uptake of basic amino acids and small peptides, but they are also used by the antibiotic meropenem for entering the cell. Carbon catabolite repression (CCR) regulates the expression of these porins by modulating the activity of the Hfq RNA chaperone, in response to the carbon sources. Hfq, in concert with the sRNA Ersa, inhibits the expression of *oprD*, while independently of ErsA can inhibit *opdP* translation. Preliminary data showed a potential interaction between the second messenger c-di-GMP and ErsA activity. The goal of this study was to evaluate whether c-di-GMP levels have an impact on meropenem resistance, and how this regulation relates to ErsA and CCR. We used strains with low, high, or physiological levels of c-di-GMP in combination with *ersA* mutation in the presence of different carbon sources to evaluate the effects that each regulatory pathway has on meropenem resistance. Our results show that low c-di-GMP levels induce meropenem tolerance. This increased tolerance is related to higher transcription of Hfq. Moreover, c-di-GMP levels can override the regulation of CCR or ErsA, as at low c-di-GMP levels bacteria are irresponsive to CCR and ErsA presence. Lastly, CCR regulation is lost in the *ersA* KO mutant, indicating that Ersa is necessary for CCR regulation. Our results show how c-di-GMP, ErsA and CCR interact in the regulation of meropenem resistance in *P. aeruginosa*.

B393

RpoS regulates the response to visible light stress in *Escherichia coli*

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Abstract

Escherichia coli can sense visible light; however, little is known about how they may respond to this stimuli. It was previously reported that treatment with visible light in the blue spectrum causes killing via the generation of reactive oxygen species. Other oxidative stresses such as, H2O2, induce the general stress response pathway (GSRP) thus, we investigated whether this was also true for blue light. The GSRP is mediated by the alternative sigma factor RpoS & when bound to RNAP, facilitates transcription of an array of 'stress response genes'. We first demonstrated that ΔrpoS was significantly more sensitive to visible (420 nm) light exposure at 14 mW/cm^2 when compared to the WT. Transcript & protein levels of rpoS/RpoS were also induced as a result of light exposure. Next, we carried out RNA-seq on irradiated cells and shortlisted genes that were significantly upregulated during light exposure, were in the RpoS regulon and were known to be involved in oxidative stress response. Consequently, we screened 23 knock out mutants for their phenotypic responses to light. A strain deficient for the DNA binding protein dps, displayed significantly increased sensitivity to light relative to both the WT and ΔrpoS. lexA and recA transcript levels were used as reporters for DNA damage. We also utilised the q-PCR DNA damage assay on DNA extracted from irradiated cells, based on the principle that presence of lesions can slow or block the progression of DNA polymerase. Light treated cells demonstrated DNA damage, which was more severe in Δdps than the WT. Dps possesses dual functions of DNA binding and iron sequestration &
when we abolished the DNA binding ability, but not the iron sequestration function, the same phenotype as Δdps was observed. These findings suggest that visible light induces DNA damage mediated by ROS stress & Dps is required to prevent this damage.

B394

Optogenetic gene expression control in *Lactococcus lactis*

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Abstract

Lactococcus lactis is a gram-positive bacterium that has been widely studied for its biotechnological and industrial applications due to its suitability to produce high-value chemicals and recombinant proteins. To this end, multiple chemically inducible gene and protein expression systems have been developed. Here we present a novel inducible system that relies solely on physical stimulus, specifically blue light, using a combination of small engineered Vivid photoreceptors from *Neurospora crassa* (enhanced Magnets or eMags) and a split T7 RNA polymerase.

Our findings demonstrate that the split T7 RNA polymerase is active and non-cytotoxic in *Lactococcus lactis*, and when fused to the eMags, it can drive gene expression in a light intensity- and time-dependent manner. This marks the first time that a split T7 RNA polymerase fused to photoreceptors has been described in a gram-positive bacterium.

This system provides an attractive alternative to chemically inducible systems since it can be activated by physical stimulus alone, thus avoiding the need to add inducers externally and improving its reversibility, without the need to remove the inducer from the medium. Furthermore, this system can be applied in various biotechnological applications where precise spatiotemporal control of gene expression is required, especially in the context of living biomaterials where chemical stimuli are diffusion-controlled, difficult to switch off and thus cannot be applied locally in a reversible way, a role where light excels. We foresee potential applications in tissue engineering and industrial protein production.

B395

Dose-dependent reaction of *Pseudomonas aeruginosa* to nanomaterials

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Abstract

Nanomaterials are often introduced as antimicrobials toxic to bacterial cells via multiple modes of action. These include the generation of reactive oxygen species by photocatalysis or reactivity of ions, mechanical interaction with cells (e.g., graphene oxide or carbon nanotubes), heat shock (in the case of magnetic particles), or delivery of antibiotics or adjuvants (e.g., via mesoporous silica). However, recent studies have shown that nanomaterials in specific concentrations can trigger an opposite reaction and stimulate bacteria. In some cases, nanomaterials can trigger primary and secondary metabolism, including the production of virulence factors. *Pseudomonas aeruginosa* is a good model for studying these effects because of its high adaptative capacity and rich secondary metabolism that produces, e.g., pigments, rhamnolipids, and alginate.

This study aimed to evaluate the effects of dosing selected nanomaterials on the physiological response of *P. aeruginosa*.

Experiments were conducted on *P. aeruginosa* ATCC 2783 exposed to variable concentrations of metal oxide nanoparticles (zinc oxide, titanium dioxide). Nanoparticles were delivered to bacteria directly or as components of metal-organic-framework structures and carbon nanotubes. A range of physiological measures have been evaluated, including secondary metabolite production, cell viability, membrane potential, gene expression, biomass concentration, biofilm production, and its morphology.

The results have shown that nanomaterial concentration is crucial for describing the physiological state of tested bacteria. Furthermore, decreased concentration could reach a point in which inhibitory action was transversed to stimulative effects, which have considerable implications for using nanomaterials as antimicrobials in medicine and biotechnology.

B396

The role of type III secretion chaperones in effector export in *Yersinia enterocolitica*

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Abstract

The critical steps of the secretion process such as the selection, recruitment, and export path of the effector proteins are still poorly understood. Prior to activation, we know that effectors are cytosolic, but we do not know how these proteins are exactly shuttled and recruited into the injectisome once secretion is initiated. In some cases, most effectors are bound to specific T3SS chaperones that are often co-expressed in the bacterial cytosol. Therefore, we are interested to know how chaperones govern effector secretion, specifically on how it enables or enhances export and why it is seem to be essential for translocation into the target eukaryotic host cell. To determine the roles of chaperones in effector export, we first looked at the overall dynamics of chaperones SycH and SycE in live *Yersinia enterocolitica*. Single particle tracking using Photoactivated Localization Microscopy (sptPALM) and Fluorescence Correlation Microscopy (FCS) were used to follow single-labeled chaperones. Our results show that the presence of their respective effector protein (YopH, YopE) slowed down the diffusion of chaperones. Our Co-IP data also confirmed strong interaction of chaperone with their effector particularly during export due to the native upregulation in the expression of T3SS components. Meanwhile, during secretion, we found that SycH diffuses faster and appears to be more dynamic compared to SycE. The slower mobility of SycE during secretion suggests formation bigger protein complexes. We also found that the affinity between chaperone and effector is independent of SctQ (sorting platform component) or the assembly of the injectisome (SctD).

B397

Physiology and biochemical capacities of anaerobic gut fungi direct their roles and interactions during the digestion of plant material

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Abstract

Anaerobic gut fungi (AGF), phylum Neocallimastigomycota, are powerful primary degraders of feed in ruminants such as cows and sheep. Together with bacteria, archaea, and protozoa, AGF form the rumen microbiome, who's digestive fermentation activity strongly affects both animal health and production greenhouse gas methane. Manipulation of the rumen microbiome activity is thus a promising avenue to reduce environmental impact of ruminant farming. However, this requires better understanding of the microbiome function, and in particular the roles of eukaryotic members.

Aiming to uncover the roles of AGF, we assessed how a panel of AGF species with distinct physiological characteristics and biochemical capacities are active during feed digestion in vitro. Looking at the characteristics of the digested feed, we identified clear differences in degradation efficiency between fungal species, as well as distinct patterns in degradation of hemicelluloses and cellulose components, and changes in material structure. These results indicate that clearly distinct niches exist for the investigated species of AGF. We are currently exploring the molecular biology and physiological aspects of the fungi that underpin these differences, using transcriptomic and proteomics approaches.

We are also investigating how these fungal niches reflect in the interactions with other microbiome partners. We recently uncovered an exciting non-canonical interaction of AGF with bacterial partners. In this cross-feeding interaction AGF did not function as primary degrader but seemed to take a role as consumer or scavenger. As the bacteria promoted fungal growth during feed degradation, this interaction has direct implications for understanding feed digestion by the rumen microbiome.

B398

Peptidoglycan in *Orientia tsutsugamushi*: new insights into cell wall biogenesis and regulation in a neglected intracellular bacterial pathogen

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Abstract

Orientia tsutsugamushi is the causative agent of the human febrile illness scrub typhus which is endemic in large parts of East Asia and the Pacific. As an obligate intracellular bacterium, *O. tsutsugamushi* has evolved in a specialist evolutionary niche in which the host immune response must be evaded and environmental osmotic pressures can fluctuate over the course of infection. This has led to a number of unique characteristics related to the synthesis and regulation of its cell wall. We show that penicillin-binding proteins (PBPs) in *O. tsutsugamushi* are either absent or have mutations in their transpeptidase catalytic site. We also explore the role that these mutated PBPs play in peptidoglycan biogenesis and in the temporal regulation of peptidoglycan throughout the infection cycle.

BLOCK B

Session : Virus Workshop: Molecular basis of the host:pathogen interaction

B400

Differential Surface Nucleocapsid Protein Immunomodulation across SARS-CoV-2 Evolution

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Abstract

We recently reported that the nucleocapsid (N) protein from highly pathogenic and common cold human coronaviruses (HCoV), a canonical intracellular antigen, is abundantly expressed on the surface of both infected and neighboring cells. We also described N as the first HCoV chemokine binding protein able to inhibit leukocyte chemotaxis.

Despite its higher antigenic stability than Spike, N is also susceptible to antigenic drift as shown across emerged SARS-CoV-2 variants of concern (VOC). N mutations R203K/G204R (KR), first appeared in the Alpha (B.1.1.7) VOC, and later in the Omicron lineage, have been reported to increase the infectivity, fitness, and virulence of SARS-CoV-2. More recently, KR mutations have been connected with an elevated inflammatory immune response in severe COVID-19 patients, compared to patients infected with SARS-CoV-2 with wild-type N. Our preliminary results show that N from the Alpha and Omicron VOC, both containing the KR mutations, have an impaired inhibitory activity of chemokine-induced leukocyte migration. We speculate that the observed hyper-inflammatory response mediated by the KR mutations in the N protein might be linked to the reduced N-induced inhibition of chemokines properties.

In context with other studies, these findings provide insights into how mutations in SARS-CoV-2 N protein modulate host immune outcome and viral pathogenesis.

B402

Emergence of Monkeypox, Polio, Influenza and Respiratory syncytial virus in wastewater: Potential for sewershed-level surveillance of viral disease

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Abstract

Viruses, both enveloped and non-enveloped, exhibit remarkable ubiquity and persistence in wastewater and receiving water bodies, which correlates with the occurrence of human infections. The introduction of these viruses into wastewater primarily occurs through the excretion of faecal matter by persons who are infected and either symptomatic or asymptomatic. The prevalence of these viruses in faecal effluents can be attributed to their ability to withstand ambient temperatures and other environmental factors. Influenza, Respiratory Syncytial Virus (RSV), Polio, and Monkeypox exhibit variations in their genome types and structures, which present unique challenges for their detection, identification, and quantification. Wastewater-based epidemiology (WBE) has emerged as a passive, comprehensive method of monitoring viral infection rates within communities. In addition to evaluating the frequency of occurrence, this system provides timely notifications of the emergence of viral variations that have significant implications for public health. This review explores the ecological dynamics, genetic characteristics, co-infection prevalence, and implications for non-clinical surveillance of viral disease. Co-infections, which are frequently observed among these viruses, are a significant problem in clinical diagnosis due to the presence of overlapping symptoms. Using wastewater-based epidemiology to study the ecology of viruses and hosts, genetic evolution, co-infection dynamics, and surveillance for re-emerging viral infections has led to important findings that can be used to better manage viral diseases.

B403

Accessory protein 3a of infectious bronchitis virus inhibits some interferon transcription factors

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Abstract

Infectious bronchitis virus (IBV) is a Gammacoronavirus which causes a highly contagious disease of domestic fowl and leads to significant economic losses in the poultry industry. The viral genome of IBV encodes for seven confirmed accessory proteins, with accessory protein 3a implicated in differentially regulating the interferon pathway. Our work aims to investigate mechanistically how this viral protein regulates interferon expression. Using transfection in Vero cells, we have demonstrated that expression of 3a results in reduced levels of interferon regulatory transcription factor IRF3. Use of immunofluorescence indicated the cytoplasmic aggregation of IRF3 with 3a expression, suggesting a dose-dependent interaction between the two. Preliminary data in chicken DF-1 cells suggested that 3a may also inhibit the expression of interferon transcription factor IRF7. Using mass spectrometry, we identified cellular interacting partners of 3a with a significant hit being CAND1. Under normal conditions, CAND1 prevents IRF7 degradation and future work will investigate the role of the 3a-CAND1 interaction in regulating IRF3/7 levels. Understanding how accessory proteins support IBV replication is a vital step forward in combating this economically important disease.

B404

Nucleotide-level conservation in measles virus--a bioinformatic approach

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Abstract

Measles (*measles morbillivirus*) was eliminated in the US in 2000 due to a successful vaccination program. The success of vaccination relies on the conservation of viral genes and low variability within the species. Previous research demonstrated conservation at the amino acid level mainly by mutagenesis approaches, showing multiple conserved loci corresponding to important protein structures. It remains unclear, however, how the genes are conserved at the RNA level. This level of conservation can yield important information about overlapping reading frames, packaging signals and other RNA motifs.

Given that measles genomes generally have low variability, a high-sensitivity algorithm described by Skittrall et. al. 2018 was used. This algorithm used weighting and ranking of all gene loci to distinguish noise (background nucleotide-level variability) from the signal (conserved regions with low variability). The rationale behind it is that some loci would be more conserved than others at the RNA level, and the longer a conserved sequence is the less likely it is background noise. There are, in total, five conserved regions found, ranging from 200 to 900 nucleotides long. One is found in the P gene, two in the H gene, one in the N gene and one in the L gene. One region corresponds to the V gene, as it has an overlapping reading frame with the P gene. The other four regions have no satisfactory explanation in the current literature, thus warrant further investigation.

B405

Characterisation of IAV inclusions in hiPSCs derived airway epithelium.

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Abstract

Influenza A virus (IAV) infections pose a significant global health threat, requiring a comprehensive understanding of the intricate host-virus interactions at the cellular level. In this regard, the airway epithelium becomes the battleground for viral invasion and cellular defense. IAV genome comprises 8 viral ribonucleoproteins (vRNPs) and it's live cycle involves several steps that are crucial for the virus to replicate and spread. Liquid condensates known as viral inclusions, concentrate the vRNPs and are viewed as sites dedicated to the assembly of IAV genome. In recent years, lung organoids derived from human induced pluripotent stem cells (hiPSCs) have been developed and successively used to study a myriad of diseases. By taking advantage of this technology
we have characterised the formation and dynamics of IAV viral inclusions within the intricate landscape of airway epithelium in a lung-on-a-chip model as was well as in Air-liquid interface cultures.

B406

Utilising human induced pluripotent stem cells (iPSCs) to investigate HSV-1 infections in neurones

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Abstract

Herpes simplex virus (HSV)-1 is a neuroinvasive human pathogen that persists in the sensory ganglia of infected hosts and can cause severe disease when it spreads to the central nervous system. Herpes simplex encephalitis is the most common viral encephalitis in the UK which, despite the availability of direct-acting antivirals, can leave patients with severe neurological sequelae. Exposure to neurotropic viruses is also increasingly associated with Alzheimer's disease and associated dementias. Induced pluripotent stem cell (iPSC) technology allows culture of authentic human neurones *in vitro*, facilitating the study of neuronal infection and providing an experimental platform for developing new prophylaxes and antiviral therapies. We have used this technology to monitor the kinetics of wild-type and mutant HSV-1 replication and neurone-to-neurone spread and have performed quantitative temporal proteomic studies to understand how wild-type and mutant HSV-1 isolates change the neuronal whole-cell and plasma membrane proteomes, plus the secretome, during infection. These -omic experiments can identify key innate immune restriction factors and other proteins of potential interest that are either upregulated or downregulated upon infection and may illuminate proteomic similarities between HSV-1 infection and neurodegenerative diseases. Preliminary analyses of these multi-omics experiments will be discussed.

B407

Functional characterisation of Wnt/wingless signalling in Rift Valley fever virus infection of mosquito cells.

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Abstract

Rift Valley fever virus (RVFV) is a mosquito-borne virus with significant implications for both animal and human health and a recognised potential to emerge globally. The Wnt/beta-catenin pathway is an evolutionarily highly conserved signal transduction pathway that regulates crucial aspects of embryogenesis, the cell cycle as well as host immune responses. We recently confirmed that the Wnt pathway facilitates RVFV replication in human cells, which is in accordance with studies showing that a diverse set of viruses manipulate and exploit the Wnt pathway for productive infection.

Interestingly, differential expression of Wnt/wingless pathway genes has also been implicated with higher infection and dissemination rates of some mosquito-borne viruses in mosquito populations. We thus sought to investigate its potential impact on viral growth in mosquito cells.

Firstly, we characterised the ability of mosquito cell lines to signal through the wingless pathway and found that all cell lines studied expressed low levels of the Wnt receptor Frizzled-2 (Fz2) and were impaired in signalling. We thus engineered *Aedes aegypti* Aag2 cells to either overexpress Fz2 or a constitutively active form of mosquito beta-catenin (armadillo). The generated Aag2 cell lines will be analysed for their signalling ability using qRT-PCR, immunoblotting and immunofluorescence techniques. We will then quantify the functional impact of Wnt signalling on RVFV growth in these cells.

This comprehensive strategy provides valuable insights into the regulatory role of the Wnt pathway in RVFV replication dynamics within mosquito cells.

B408

Barriers against Zoonosis: Defining Species-specific Restriction Factors against Coronaviruses

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Abstract

The pandemic nature of Coronaviruses (CoVs) is underpinned by their ability to jump from one species to another. However, such events require the virus to overcome barriers caused by evolutionary differences between host species. One such barrier is the IFN response, which is highly divergent among different species. Systematic analysis using arrayed human IFN-stimulated gene (ISG) expression libraries has been effective at identifying antiviral factors against a broad range or specific viruses. However, majority of these studies were focused on humans and libraries for other species are lacking. Pigs are important livestock species in economic and public health aspects as they are host for influenza virus and at least six CoVs. We have developed an arrayed lentiviral-based porcine Type-I ISG expression library comprising 432 ISGs to enable cross-species screens. To identify species-specific CoV restriction factors, we have applied the library to porcine respiratory coronavirus (PRCV), a highly adapted endemic CoV causing pneumonia in pigs resembling SARS in humans and SARS-CoV-2, which can replicate efficiently in some porcine cell lines but is unable to infect pigs. Using a direct lysis RT-qPCR-based protocol, we have identified novel porcine ISGs that restrict both PRCV and SARS-CoV-2 replication. We have also identified ISGs that restrict SARS-CoV-2 to a greater extent than PRCV, suggesting SARS-CoV-2 is less adapted to the porcine IFN response, which may contribute to barriers against *in vivo* infection. These studies show that comparative ISG screens against endemic versus non-adapted viruses are effective in identifying specific-specific and novel host restriction factors.

B409

Forecasting the next panzootic foot-and-mouth disease virus lineage: informing virus fitness from in vitro studies and genomic data.

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Abstract

Foot-and-mouth disease virus (FMDV) is the causative agent of FMD, a highly transmissible disease affecting cloven-hoofed animals. FMD has a high economic burden affecting trade globally both in endemic and non-endemic countries. FMDV is divided into genetically distinct topotypes and lineages based on geographic location and nucleotide divergence. Whilst new lineages of FMDV often emerge, only a subset proceed to become the dominant circulating variants. These lineages outcompete and replace the circulating lineages in or beyond a geographic area in a process known as lineage turnover. In this project we investigate whether there are genome-encoded viral fitness factors that contribute to these lineage turnover events. Twelve FMDV isolates representing four panzootic and non-panzootic lineages within serotype O were selected for genomic and *in vitro* phenotypic characterisation. Growth and plaque phenotypes were characterised in porcine kidney cells. Variation in plaque size among isolates correlated with differences in the onset of cytopathic effect (CPE) observed in growth curve analyses. Notably, isolates SRL/01/2019 and UAE/14/2021 showed a delay in the onset of CPE and produced conspicuously smaller plaques compared with other isolates such as VIT/31/2019 and SAU/09/2018. These studies were then repeated in primary cells isolated from FMDV-susceptible species to represent a better *in vitro* model of the natural infection. These phenotypic data are presented in conjunction with sequence data to identify viral determinants of replicative fitness. Together, these results will help to provide an understanding of virally encoded factors underpinning the emergence and spread of panzootic lineages.

B410

Rationally designed live attenuated influenza vaccines: Impact of influenza A virus synonymous genome recoding on viral gene expression

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Abstract

Development of new rationally designed live attenuated influenza virus vaccines could confer safety, efficacy, and logistical advantages over currently used vaccine platforms. The antiviral protein ZAP binds CpG dinucleotides and we are analysing the effect of synonymously recoding IAV genome segments by introducing this motif in different contexts to determine if it consistently leads to ZAP-mediated viral restriction. We examined the effects of an algorithm-guided CpG- and A-enrichment of IAV segments 2 and 4 by analysing their impact on protein expression using a mini-replicon assay. Unexpectedly, CpG recoding of segment 2 increased PB1 expression, potentially due to the elimination of cryptic splice sites. HA expression from the recoded segment 4 was decreased in a ZAP-independent manner, possibly caused by de-optimised codon or di-codon usage. A viral rescue experiment demonstrated approximately 10,000-fold attenuation of viral replication for the segment 4 recoded virus. These results show that synonymous genome recoding of influenza A virus by introducing CpG dinucleotides and increasing the adenosine content can have variable effects and does not always lead to ZAP-mediated inhibition. Further understanding of the nucleotide determinants that are required for ZAP to restrict negative sense viral replication as well as potential viral countermeasures will allow the generation of reproducible synonymous genome recoding strategies.

B411

Influenza A virus protein NS1 is insoluble in the cell

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Abstract

Solubility proteome profiling distinguishes soluble and insoluble components within the cellular environment. Different factors contribute to protein transitioning into an insoluble state, including binding to membranes, forming complexes, undergoing liquid-liquid phase-separation, gelation, or precipitation. Additionally, protein localization to specific organelles can lead to insolubility. To understand the physiological alterations associated with changes in protein abundance and solubility during influenza A virus (IAV) infection, the first step was to analyse the proteome abundance and solubility profiles of IAV-infected cells at various times post-infection. We identified 6,629 proteins, 184 exhibiting changes in abundance and 413 in solubility. Solubility changes in cellular proteins correlated with mitochondria, translation, and processes associated with liquid-liquid phase separation (stress granule formation, nucleoli, splicing). Regarding viral proteins, three components underwent phase transitions, becoming insoluble during infection: viral ribonucleoproteins (vRNPs), non-structural protein1 (NS1), and neuraminidase. While the cytosolic accumulation of vRNPs in liquid viral inclusions was previously documented and neuraminidase is a transmembrane protein, NS1 is distributed throughout the cell and its insolubility was not reported before. The following step was to validate, for several proteins, solubility changes through immunofluorescence and westernblotting after NP40 (soluble) or SDS (total proteome) treatments. To understand the physiological relevance, we concentrated in NS1 increased insolubility. Our findings reveal that NS1 accumulates in non-spherical, punctate structures in the cytosol, potentially resulting from its interaction with cellular RNA. Our work opens avenues to explore how modifications in the material properties of cellular/viral components influence cellular functions, contribute to viral infection and host defences, and may identify targets for innovative antiviral strategies.

B412

Characterizing the Role of Glycosaminoglycans in a Novel Lyssavirus Infection

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Abstract

Lyssaviruses are a genus of RNA viruses that can cause disease in humans and are often associated with neurological pathogenesis that can be fatal. A novel lyssavirus (LYSV) has recently been identified in *Pipistrellus abramus* (Japanese pipistrelles) in Asia. Although infections in humans have not yet occurred, there is a risk that this species could become an emerging virus for animals and humans. The aim of our study is to investigate virus-host interaction, particularly at the cell entry level. Our focus is on the interaction between the viral glycoprotein G and the attachment factor glycosaminoglycans (GAGs) on the cell surface. We cloned and established LYSV pseudoparticle models and assessed their infectivity on human brain cells, the primary target of lyssavirus infection. To confirm the role of GAGs in LYSV infection, we infected the cells in the presence of soluble heparin to compete with GAG binding or used enzymes to remove the GAGs on the cell surface. The results show that soluble heparin impaired LYSV infection in a dose-dependent manner, and that removal of GAGs also affected LYSV infectivity. Different desulfated forms of heparin also showed strong inhibition on LYSV infection. Next, to identify potential molecular determinants of LYSV G binding, a homology model was constructed, and several candidate GAG-binding residues were identified. Work is underway to elucidate their role in LYSV G-GAG interaction via mutagenesis analysis. In summary, this study reveals cell surface GAGs as an attachment factor for mediating LYSV infection in human brain cells.

B413

Human Circulating Macrophage Inflammatory Protein-1 Alpha: A Novel Prognostic Marker of Hepatocellular Carcinoma in Chronic Hepatitis B Patients

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Abstract

Backgrounds: Chronic hepatitis B infection results from the interplay between the HBV and the host immune response. Therefore, the stages of infection are determined by the liver inflammation. This study aimed to determine a novel inflammatory prognostic marker of liver cirrhosis in chronic hepatitis B patients.

Methods & Materials: Chronic hepatitis B patients with non-cirrhotic, cirrhotic and acute flare infections were recruited. The control group were recruited from healthy undergraduate students of the Faculty of Medicine and Health Sciences Universiti Putra Malaysia that were HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc negative. A total of 40 human cytokines and chemokines were screened using magnetic beads multiplexed Luminex assay. The plate was read immediately using the Luminex analyser (Luminex Corp. Texas, USA).

Results: Human Interleukin-1 receptor antagonist (IL-1ra) was found to be positively correlated with liver cirrhosis ($r=0.2066$; $p<0.05$) and acute flare ($r=0.2258$; $p<0.05$) while in non-cirrhotic patients was found to be negatively correlated ($r=0.3938$; $p<0.05$). Similarly, IL-1ar was also found to be significantly associated with Elevated ALT ($r=0.268$; $P<0.05$), and AST levels with IL-1ar ($r=0.257$; $P<0.05$). IL-1ra is secreted by immune system cells secreted by the liver, it is considered an acute phase reactant that functions as proinflammatory cytokine by stimulating natural killer cells.

Conclusions: It has been shown that knocking down of IL-1ra in mice decreased liver inflammation therefore, its detection of IL-1ra in chronic hepatitis B infected patients might serve as a novel prognostic factor of cirrhosis and acute flare.

B414

Identification of neutralising nanobodies to the conserved S2 region of beta-coronavirus spike proteins

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Abstract

Nanobodies are single domain antibodies, derived from the variable domains of Heavy Chain only antibodies of camelids. We are investigating the potential of nanobodies as therapeutics against a broad range of beta-coronaviruses by targeting the S2 region of the spike protein which is highly conserved between different viral subgroups. From a nanobody library prepared from llamas immunised with the spike proteins of three different coronaviruses viruses (SARS-CoV-2, HCoV-OC43 and MERS-CoV), we have identified binders to the spike S2. The selectivity profiles of the nanobodies for different viral spike proteins have been assessed by ELISAs, binding affinities by Bio-layer interferometry and functionality investigated in live virus neutralisation tests. Single particle cryo-electron microscopy is being used to map the binding locations of the nanobodies on the SARS-CoV-2 spike protein providing insights into their mechanism of action. We will discuss the potential of nanobodies that target the S2 region of spike proteins, for developing cross-protective therapeutics against current and possibly future pathogenic coronaviruses.

B415

Alkyne Derivatives of SARS-CoV-2 Main Protease Inhibitors Efficiently Inhibit Isolated Mpro and SARS-CoV-2 Replication in Cells.

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Abstract

Effective vaccines to combat the SARS-CoV-2 virus have been successful in decreasing hospitalisations and mortality in many countries. However, as has been seen with the emergence of new variants of concern (VOC), vaccine breakthrough is a regular occurrence. Additionally, individuals with immune deficiencies are not effectively protected by vaccines. Therefore, there is an obvious need for the development of effective antivirals. First generation antivirals such as remdesivir require intravenous administration in a hospital setting and have a short half-life. The development of oral drugs such as Molnupiravir received regulatory approval in the UK in Nov 2021 but have since been shown to have limited efficacy in disease settings.

In late 2021, the second generation, small-molecule active pharmaceutical ingredient of paxlovid, i.e., nirmatrelvir was approved for emergency use in humans to treat COVID-19 (in combination with ritonavir). Nirmatrelvir is a nitrile-bearing small-molecule inhibitor that acts to interrupt the viral life cycle by inhibiting the SARS-CoV-2 main protease (M^{pro}), which is essential for processing viral polyproteins into functional non-structural proteins.

M^{pro} is highly conserved among coronaviruses, including SARS-CoV-2 variants of clinical concern.

We have synthesised derivatives of nirmatrelvir and other MPro inhibitors, including an irreversible alkyne covalent SARS-CoV-2 MPro inhibitor, and tested their effects on SARS-CoV-2 replication in-vitro. With inhibitory EC50 values (≥ 5.1µM) approaching that of electrophilic nitriles such as in nirmatrelvir, there is therapeutic potential for the covalent inhibition of M^{pro} and other nucleophilic cysteine proteases by alkynes.

B416

Splice to Survive: Diversification Strategies of Interferon-stimulated Genes

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Abstract

The interferon response is the first barrier against virus infections. Interferon-stimulated genes (ISGs) are under constant selection pressure imposed by viruses they inhibit, resulting in an enhanced frequency of gene duplication in ISG families relative to other genes. For instance, the interferon-induced transmembrane proteins (IFITM) are a family of antiviral ISGs that has undergone repeated species-specific duplication events. Yet, gene duplication is not the only evolutionary means of generating diversity. Here, we show that alternative splicing leads to functional expansion of the IFITM family using horseshoe bats as an exemplar. Alternatively spliced IFITM isoforms of Chinese horseshoe bats are expressed at different levels in native cells and exhibit differential antiviral potency, suggesting that alternative splicing is a means for functional diversification. *In silico* analysis of *IFITM* genes in 206 mammalian species reveals that *IFITM* alternative splicing is evident in 36% of mammals and is strongly associated with *IFITM* gene duplication. This demonstrates that alternative splicing is a ubiquitous yet underappreciated strategy for ISG diversification in addition to gene duplication. We further extend our analysis to a wider pool of ISGs for a more holistic understanding of their evolutionary strategies. These findings showcase an example of convergent evolution where expansion of ISG repertoires is achieved through multiple means, highlighting the importance of ISG diversity in host innate immunity. Our data provides a framework for selecting ISGs to further characterise based on their evolutionary dynamics across species, which may have implications on their role in zoonotic barriers.

B417

Investigating CoV spherules using a replicon-based system

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Abstract

Coronaviruses, like other +ssRNA viruses, form replication organelles within infected cells to facilitate efficient RNA synthesis and to avoid host detection. Coronavirus replication organelles are comprised of double membrane vesicles (DMVs), zippered ER and double membrane spherules. DMVs are known to be the site of viral RNA synthesis. However, although spherules will be complex and high-energy to produce, their exact function remains unknown. As formation of replication organelles is conserved across the whole virus family, a detailed understanding of their formation and function may inform future development of broad novel control strategies. In order to study Coronavirus spherules, we aim to identify the viral proteins responsible for their formation. Replicon-based expression cassettes have been generated for avian *Gammacoronavirus* Infectious Bronchitis virus (IBV). These express defined regions of the IBV genome, each with a GFP-tagged protein to allow direct visualisation. Initial experiments indicate that expression of replicase gene 1a (Nsps 2-10) produces all the required proteins for replication organelle formation, as determined using immunofluorescence. Ongoing work aims to optimise efficiency of protein expression from these cassettes and to determine the membrane rearrangements associated with expression of this subset of viral proteins.

B418

Understanding proteolysis during Zika virus infection

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Abstract

Zika virus (ZIKV) is a vector-borne Orthoflavivirus, endemic to 89 countries worldwide, with infection linked to neurodevelopmental impairment in neonates as well as Guillain-Barré disorder in adults. Currently there are no approved anti-virals for treatment. However, a key element of ZIKV replication, the viral protease non-structural protein 3 (NS3), has been established as an attractive target for this purpose. NS3 processes the translated viral polyprotein, but potential interactions with the host has not been fully explored. Previous work characterizing ZIKV NS3 activity using N-terminomics in a non-infection context was reported by Hill et al., 2018. Here, we are expanding on this research in an infection context using mammalian cells to provide a thorough interrogation of activity using N-terminomic analysis (LC-MS) to identify and quantify cleavage sites in both viral and cellular substrates. In this poster we present our preliminary findings of ZIKV cleavage in A459 cells, and plan to expand this work to insect cells.

B419

Elucidating Influenza A virus genome packaging interactions in the context of infection

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Abstract

The segmented nature of the influenza A virus (IAV) genome provides it with an evolutionary advantage, allowing for reassortment of virus segments upon co-infection of differing strains. It also poses a problem: the virus must ensure that one of each of its eight segments are bundled and packaged to produce an infectious virion. These segments are organised as viral ribonucleoprotein complexes (vRNPs), which consist of viral RNA (vRNA) bound non-uniformly to nucleoprotein, allowing for the formation of RNA secondary structures. Work from our lab has shown that these structures facilitate vRNA-vRNA interactions within virions. Alongside other reports showing co-localisation of vRNPs post nuclear export, this work suggests a potential role for vRNA-vRNA interactions in segment bundling.

Here, we report on work to elucidate the IAV genome assembly pathway during infection, using biochemical methods, structural sequencing techniques, and RNA fluorescent *in situ* hybridisation (FISH). Following cellular fractionation and immunoprecipitation, we have employed selective 2'-hydroxyl acetylation analysed by primer extension and mutational profiling (SHAPE-MaP) to probe local RNA structure, and sequencing of psoralen-cross-linked, ligated and selected hybrids (SPLASH) to probe long-range vRNA-vRNA interactions. Through comparison of this data over the course of infection to that from purified virions, we identify key inter-segment interactions that drive the genome bundling process. Future work will focus on the effects of co-infection with different IAV strains. We hope to use this information predict the likelihood of IAV strain reassortment and provide insight into the processes that underpin pandemic strain emergence.

B420

Exploring the Interactions of Single-Stranded DNA Molecules with Divergent Coronavirus Structural Proteins: ELONA and Flow Cytometry

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Abstract

The ongoing global challenges posed by various coronavirus strains necessitate a deeper understanding of the molecular interactions between viral proteins and alternate-binding molecules. This study focuses on the interplay between single-stranded DNA (ssDNA) molecules and divergent structural proteins of coronaviruses. The investigation employs Enzyme-Linked Oligonucleotide Assay (ELONA) and Flow Cytometry to analyse these interactions.

ELONA allows for the precise detection of molecular interactions by leveraging the binding affinity between ssDNA and coronavirus structural proteins. This method is complemented by Flow Cytometry, providing a quantitative and dynamic assessment of the interactions in a high-throughput manner. Divergent structural proteins from various coronavirus strains are expressed and purified to ensure a representative sample for analysis.

Preliminary findings reveal distinct binding patterns between ssDNA molecules and coronavirus structural proteins. ELONA data highlight specific interaction regions, shedding light on potential charge-based nucleic acid binding domains within the viral proteins. Flow Cytometry analyses further elucidate the kinetics and quantitative aspects of these interactions.

The observed diversity in ssDNA binding across various coronavirus strains underscores the need for an improved understanding of binding interactions for implications for antiviral strategies and diagnostic applications targeting crucial structural proteins. Our results contribute to a deeper understanding of alternate molecular tools to visualise and understand viral replication and pathogenesis, potentially informing the development of more comprehensive bio-veterinary research tools.

B421

Characterising the molecular determinants in emerging SADS-CoV regulation of stress signaling in bats to understand its cross species transmissibility

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Abstract

Coronaviruses have the ability for cross-species transmission and hence pose a great threat to both human and animal health through emergence of novel viruses. The intracellular virus-host interactions involved in coronaviruses cross-species transmission are not well characterised. Swine acute diarrhea syndrome coronavirus (SADS-CoV) is a highly virulent emerging porcine coronavirus, closely related to bat coronavirus HKU2. SADS related (SADSr)-CoVs isolated from bats with intermediate greater sequence homology to SADS-CoV have also been identified. The spike gene and the viral accessory genes are the regions of the genome exhibiting greatest sequence variation between the bat and porcine viruses. Therefore, in this work, we aim to investigate the role of viral accessory gene-mediated regulation of bat and porcine antiviral interferon and stress signalling pathways in coronavirus inter-species transmission.

Expression of viral proteins has been optimised and reagents validated to study stress granule formation in bat cells by immunofluorescence. On-going work is characterising in more detail the stress signalling pathway in bat cells as well as the regulation of stress signalling by viral proteins in bat and porcine cells. Finally, ability of viral proteins to regulate interferon signalling in bat and porcine cells is being investigated using reporter plasmids. Advancing the characterisation of the viral regulation of interferon and stress responses in different species will support better prediction of host switching events and control of potential future outbreaks.

B422

Correlative cryo-bioimaging to study coronavirus replication organelles

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Abstract

The *Gammacoronavirus* Infectious Bronchitis Virus (IBV) is a highly contagious pathogen of poultry and can be used as a model coronavirus (CoV) to study the formation and structure of CoV replication organelles (ROs). Conserved RO structures exist including double membrane vesicles (DMVs) and double membrane spherules (DMSs). The mechanism of DMV formation and the function of DMSs have not yet been elucidated. Using fluorescent recombinant viruses and correlative cryo-bioimaging, the location of RO membranes will be highlighted so that they can be directly targeted for ultrastructural interrogation. These techniques will reveal ROs in 3D, to high resolution, and under near-native conditions. Fluorescent tags were inserted at the N-terminus of nonstructural protein 2 (nsp2) using reverse genetics and confirmed to mark sites of nascent viral replication and ROs using immunofluorescence imaging and room-temperature electron microscopy. Plunge freezing was used to make cryo-preserved samples for cryo-correlative light and X-ray tomography (cryo-SXT, B24, Diamond Light Source) and cryo-correlative light and electron tomography (cryo-ET, eBIC). Recombinant GFP-nsp2-IBV does not significantly attenuate viral replication and colocalises with sites of nascent viral RNA synthesis and known RO structures. Correlative cryo-SXT has revealed RO networks in whole cells to 30nm resolution. Plus, correlative fluorescence, focused ion beam (FIB) milling, and cryo-ET has resolved the structure of the DMS bilayer. Sub-volume averaging on cryo-electron tomograms will gives clues to the RO protein composition and their functions. This work will significantly improve our knowledge about the sites of CoV RNA synthesis and this critical stage of the virus lifecycle.

B423

Development and application of a novel siRNA library for the screening of porcine coronavirus host-pathogen interactions

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Abstract

RNA interference (RNAi) screening offers a valuable model to study host-pathogen interactions. Identification of pro- and anti-viral host factors informs our understanding of infection pathobiology, the host immune response, and therapeutic targets. RNAi screening has identified host factors for a wide range of human pathogens. However, as siRNA libraries are limited to a narrow species range and with no open access tools for library development, this technology has yet to be fully harnessed for other important pathogens, including those of livestock species. Here, we design and validate a porcine druggable genome siRNA library, applying it to screening host factors involved in porcine coronavirus infection.

We developed a bioinformatic pipeline and designed siRNAs for 6990 genes, scoring and selecting sequences based on defined criteria and subsequently applying BLAST analysis to filter sequences based on off-targets within the porcine genome. The influence of oligo purification, concentration, and 3' modification on gene silencing was studied across a selection of siRNAs. We applied our novel siRNA library to high-throughput screening of *Alphacoronavirus* transmissible gastroenteritis virus host-pathogen interactions, sharing preliminary results. Our novel application of RNAi screening to porcine coronavirus infection could help prepare the agricultural and veterinary sectors for future coronavirus emergences and epizootics. Furthermore, our library is applicable to studying not only host-pathogen interactions, but any quantifiable phenotype. Finally, our pipeline could be applied to develop siRNA libraries for any annotated genome, enabling the application of RNAi screening to a range of species without commercially available alternatives including economically valuable livestock animals.

B424

Development of a CRISPR/Cas9 GeCKO library screen to identify pro-viral cellular genes in chicken cells that can be exploited to control avian endemic viruses

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Abstract

In the United Kingdom, infectious bronchitis virus (IBV) and infectious bursal disease virus (IBDV) are endemic and pose a significant threat to the poultry industry and food security. Given the shared characteristics in their replication cycles, such as the possession of RNA genomes, entry via endocytosis, and replication within the cytoplasm, the objective of this project is to identify host factors that are commonly involved in the host-virus interactions, utilizing a genome-scale CRISPR/Cas9 knockout (GeCKO) screen. The screening strategies to investigate IBV and IBDV infections, including flow cytometry-based and cell survival-based screening, were developed and optimized for chicken fibroblast DF-1 cells, a cell-line that supports the replication of both viruses. Cells with a desired phenotype (e.g. resistant to virus replication) were collected for DNA extraction and next-generation sequencing. A list of genes was identified from the screens and 3 candidate genes, including cadherin-2 (CDH2), were selected for downstream characterisation. DF-1 cells with knockout of target genes were generated and virus replication ability was assessed. Host-virus interactions were then evaluated using western blot, confocal microscopy and RT-qPCR. The findings will improve our knowledge of the replication mechanisms of IBV and IBDV, and the genes identified may be exploited to produce virus-resistant chickens in the future.

B426

Characterizing rotavirus genetic composition for optimal gene expression and translation

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Abstract

Viruses typically mimic their hosts' genetic composition, such as nucleotide, dinucleotide, and codon usage. However, how these composition traits affect transcription and translation efficiency of dsRNA viral genes is not well characterized. As a leading global pathogen of diarrhoea-associated mortality, the dsRNA virus rotavirus accounts for ~128,500 deaths annually and poses a global health burden in all age groups despite available vaccines. To investigate the coding preferences of rotaviruses, we are utilizing a GFP library comprising 195 synonymously recoded GFP sequences that exhibit wide variation in nucleotide, dinucleotide, codon, and codon pair usage. These GFPs are being tagged onto the C-terminus of simian strain of rotavirus (SA11) NSP3 protein with a 2A linker for enhanced expression. Our preliminary data with a subset of eight SA11-GFP viruses demonstrate a modest effect of GFP composition on viral replication kinetics, but a more profound effect was seen on GFP production. While some constructs demonstrate significantly different fluorescent signal over time, there is no correlation between virus replication and GFP production. Utilizing the whole library will enable the identification of compositional parameters relevant for the optimal gene expression and protein production during rotavirus infection, and further characterization of how they influence polymerase activity, RNA degradation rate, protein synthesis and protein degradation rates, as well as host responses. Synonymous recoding of the RNA virus genome could be a powerful tool for augmenting existing live-attenuated rotavirus vaccines.

B427

Two cellular AAA+ ATPases associate with rhinovirus non-structural proteins and are essential for rhinovirus replication

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Abstract

Rhinovirus (RV) infections cause common colds and are a major trigger for acute exacerbations of asthma and chronic obstructive pulmonary disease (COPD). However, there are no approved vaccines or antiviral drugs against RVs. During infection, RV non-structural proteins (NSPs) interact with cellular proteins to subvert host cells and facilitate viral replication. An antiviral strategy, which is less likely to lead to the emergence of resistance than directly targeting viral proteins, is to interfere with these cellular proteins hijacked by RV.

While some of these proviral cellular proteins are known, others likely remain undiscovered. To identify new NSP-interacting cellular targets, we infected HeLa cells with RV-A16 and pulled-down NSPs and their interactors by NSP-specific affinity purification. Analysis of the pulled-down proteins by quantitative mass spectrometry revealed a specific enrichment in known proviral host factors (GBF1, PI4KIIIβ, SETD3…), along with two AAA+ ATPases that were not previously shown to be involved in the replication of RV or any other picornavirus. Remarkably, we found that their knockdown drastically inhibits RV replication, while a small molecule inhibitor of these ATPases efficiently blocks the replication of RV species A and C in cell lines and in well-differentiated primary nasal epithelial cell cultures without cytotoxicity. Together, our data show that these ATPases represent novel host factors that are essential for RV replication, most likely at an early stage of the viral replication cycle and independently of cellular transcription. In turn, this highlights their potential as promising drug targets for the treatment of RV infections.

B428

In-depth analysis of murine norovirus infection by LC-MS/MS

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Abstract

Murine norovirus (MNV) grows readily within tissue culture making it an ideal model system to study norovirus biology, given the challenges of studying human norovirus. However, limited work has been carried out exploring the profile changes of both the viral and host cellular proteomes during infection. Here we explore the proteome of virally-infected BV-2 cells over a duration of 12 hours using DIA-based LC-MS/MS. Here we present our preliminary analysis of a two-hourly infection time course and compare with prior published data. Infections were performed at a MOI of 2 to ensure the time course represented both infected and bystander cells. Ultimately, we plan to contrast this data with single-cell proteomic analysis to understand cell-cell variation in virus replication, and virus-host interactions and immune responses.

B429

Adherence of monocytes to the endothelium is enhanced by the presence of SARS-CoV-2 accessory protein ORF7a

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Abstract

SARS-CoV-2 still poses a major problem for at risk individuals with COPD or immunodeficiencies and is speculated to interact with the endothelium though an unknown mechanism. The viral accessory protein ORF7a has been predicted to facilitate viral-leukocyte interactions due to structural homology with ICAM-1 and to its binding affinity to the MIDAS site found on Mac-1. We, therefore, tested the hypothesis that ORF7a could facilitate increased adhesion of human monocytes to endothelial cells.

The effect of ORF7a was tested *in vitro* using a monocyte-endothelial cell adhesion assay. HUVECs were seeded in 96-well plate, and either the HUVECs or monocytes were exposed to concentrations of ORF7a or TNFa. After 4 hours, monocytes were tagged with calcein-AM and incubated with HUVECs for 2 hours. Cell binding was then quantified by fluorescence.

We found that monocyte adherence was increased by 36.5±4% compared to unstimulated cells when HUVECs were exposed to 3nM ORF7a for 4 hours. Monocytes exposed to 300pM ORF7a saw a similar 27.2±7% increase after 4 hours, a 10-fold lower peak compared to their HUVEC stimulated counterparts. ORF7a also induced significant expression of several proinflammatory cytokines including IL-6, CXCL1, and CCL2 saw significant increases in secretion in monocytes.

These results suggest that ORF7a facilitates the binding of monocytes to endothelial cells and to enhances the pro-inflammatory binding induced by TNFa. This provides a mechanism through which SARS-CoV-2 can cause inflammation and indicates how low levels of circulating virus in PASC may lead to chronic inflammation in patients.

B430

Highly-stable cell lines for virus propagation using the Sleeping Beauty transposon system

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Abstract

Generation of cell lines that overexpress proteins required to support virus propagation are invaluable for virology research. Traditional methods for their production usually use lentiviruses which are able to integrate DNA into a host cell genome. However, these vectors have a limited transgene size, and the transgene insertions may be unstable due to retrovirus silencing. Here, we utilise the Sleeping Beauty (SB) transposon which is a non-viral vector that mediates the integration of a gene of interest into mammalian cell lines with high efficiency.

Cell lines used for propagation of viruses typically express cellular receptors or other host factors necessary for viral replication. The glycoproteins spike (S) and haemagglutinin (HA) from SARS CoV-2 and Influenza A respectively, rely on proteases for cleavage in order to acquire membrane fusion competence which is an essential part of the viral lifecycle. One such protease, TMPRSS2, has been implicated in the cleavage activation of both glycoproteins. We therefore took existing cell lines used for influenza A (MDCK, MDCK-SIAT1 cells) and SARS-CoV-2 (VeroE6) propagation and generated versions overexpressing human TMPRSS2. Cell lines were produced rapidly and validation of transgene insertion were carried out using flow cytometry. We show that the MDCK-hTMPRSS2 and MDCK-SIAT1-hTMPRSS2 cells support Influenza A replication in the absence of exogenous trypsin. Future work includes generating permissive cells lines for the seasonal coronavirus OC43, as well as developing reporter cell lines that fluoresce upon viral infection.

B431

Dual Antigen Presentation VLP Technology using Affimer and SpyTag Capture for Vaccine Development

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Abstract

Virus-like particles (VLPs) represent promising and versatile options in vaccine development due to their safety and high immunogenicity. These non-infectious structures mimic native viruses and effectively stimulate immune responses. VLPs produced in yeast, bacteria, insect, or mammalian cells can present a variety of antigens, targeting numerous pathogens.

Our research introduces a novel presentation VLP platform based on a fused dimer of the hepatitis B core (HBc) antigen, incorporating two antigen-capturing systems: Affimer and SpyTag. The Affimer is engineered into the major immunodominant region (MIR) and the SpyTag into the N-terminus, allowing the modified VLPs to, simultaneously, present multiple antigens. These VLPs were produced in a yeast expression system *(Pichia pastoris)* and demonstrated structural integrity and correct assembly.

These VLPs can be decorated with different antigens using both capturing methods (Affimer and SpyTag). This ability to use two systems for binding antigens greatly improves the usefulness of the VLPs as flexible vaccine platforms. Being able to present several antigens simultaneously might result in better vaccines.

Current efforts are focused on evaluating the immunogenicity and efficacy of these VLPs. Our aim is to push the boundaries of vaccine development, offering new solutions for emerging infectious diseases. This dual-antigen capturing approach could provide a robust platform for developing next-generation vaccines.

B432

Illuminating the impact of proteome-wide SUMOylation on human cytomegalovirus replication

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Abstract

During viral infection, many aspects of the host molecular machinery are manipulated to evade the hostile cellular environment and allow successful replication. Post-translational modification (PTM) of proteins is also included in this, with a diverse array of viruses hijacking many different pathways. Human cytomegalovirus (HCMV) is known to take advantage of cellular SUMOylation pathways, with key viral transactivators IE1/IE2 and viral polymerase UL44 regulated by the addition of SUMO1. However, there is a lack of information on the effect of global cellular and viral SUMOylation patterns on HCMV infection, particularly with regards to SUMO2/3 conjugation, which primarily occurs during cellular stress.

Analysis of global SUMOylation patterns shows a strong increase in SUMOylated proteins during late stages of viral replication, which was absent in non-permissive cell lines, suggesting that HCMV infection elicits major SUMOylome changes. Utilising the SUMO E1 inhibitor TAK-981 revealed lifecycle stage-dependent requirements for SUMOylation during HCMV infection, with treatment during early stages of infection demonstrating a pro-viral effect, but at later stages TAK-981 powerfully inhibits replication. This early pro-viral phenotype was only partially dependent upon PML, implicating additional SUMO-regulated processes in restriction of viral gene expression. Global analysis of native SUMO2/3-modified proteins by quantitative mass spectrometry will be employed to comprehensively characterise changes in the cellular and viral SUMOylome during HCMV replication, to reveal novel cellular pathways regulated by SUMOylation that are manipulated by HCMV.

B433

Non-canonical amino acid incorporation into HIV-1 to study the role of the capsid during infection

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Abstract

The human immunodeficiency virus (HIV) capsid is essential for productive infection. It is composed of repeating units of capsid protein (p24) which form a cone of predominantly hexamers and exactly 12 pentamers. Upon receptor binding and membrane fusion, the capsid core is deposited into the cytosol and remains intact until uncoating immediately prior to integration in the nucleus. The capsid is known to be essential for microtubular trafficking towards the nucleus, shielding viral RNA from cytosolic pattern recognition receptors and as a reaction vessel for reverse transcription (RT). However, exactly how the capsid is transported across the nuclear pore complex and how nucleotides enter the capsid to drive DNA synthesis is unclear. To gain more insight into capsid function, we have used an efficient amber suppression system to incorporate non-canonical lysine derivatives into different positions within capsid, concentrating on positions which lie within the central pore of capsomers. We have confirmed incorporation into p24 with no obvious assembly or maturation defects and produced pseudotyped viruses that show reduced infectivity to wildtype. Preliminary data suggests all non-canonical lysine incorporated viruses have reduced RT, consistent with the importance of maintaining capsid pore integrity. Alongside the introduction of point-mutations, the ability to incorporate non-canonical amino acids with different properties into retroviral proteins like capsid could be an important tool for gaining greater understanding of the infectious virus cycle.

B434

Neuraminidase-dependent entry of influenza A virus is determined by haemagglutinin receptor-binding specificity

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Abstract

Influenza A viruses (IAVs) contain sialoglycan-binding haemagglutinin (HA) and sialoglycan-cleaving neuraminidase (NA) proteins, the concerted action of which is required for escape from decoy receptors and for virion motility, ultimately resulting in infection of epithelial cells of the respiratory tract. The importance of NA in egress of newly assembled virions has been well established, whereas its role in entry has yet to be fully elucidated. In this study, we systematically analysed the role of NA in viral entry in relation to HA receptor-binding preference, the receptor repertoire displayed on cells and the presence of mucus decoy receptors. Utilising recombinant viruses that differ only in their HA-NA composition, it was observed that the dependence on NA activity for IAV entry is greatly determined by HA and not NA, with entry of α2–6 sialoglycan-binding viruses being inhibited more by NA inhibitor oseltamivir carboxylate (OsC) than α2–3 sialoglycan-preferring viruses. In agreement with this, inhibition of virus entry by OsC could be modified by altering the sialoglycan receptor repertoire of cells. Entry inhibition by OsC correlated with the ability of mucus to inhibit infection, with the combination of the two having the largest effect. Our results indicate that the dependency of IAV on NA activity and, thus, virion motility for entry is determined by the receptor-binding properties of HA together with the receptor repertoire present on cells. This dependency is greater when fewer preferred receptors are displayed, which coincides with increased inhibition by mucus decoy receptors.

B435

Understanding the Molecular Mechanisms Underlying Enhancement of Influenza A Virus Infectivity by a Co-Infecting Bacteria *Klebsiella pneumoniae*

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Abstract

Influenza A virus (IAV) is a major respiratory pathogen of humans and other animals, and is a cause of yearly epidemics, and frequent pandemics. IAV infects the epithelial cells lining our respiratory tract where it can efficiently transmit between individuals, and cause life-threatening disease. During infection of the respiratory mucosa, IAV can interact with members of our microbiome and/or co-infecting pathogens, such as the gram-negative pathogen *Klebsiella pneumoniae*. These co-infections are common and are associated with enhanced disease and challenges in clinical management, partly driven by increasing antimicrobial resistance. Novel interventions targeting co-infections may be of clinical utility. However, the molecular mechanisms controlling co-infections are poorly understood.

To investigate the molecular and cellular biology of co-infections, we established a tractable *in vitro* model of IAV and a hypervirulent strain of *Klebsiella pneumoniae* co-infection of human alveolar epithelial A549 cells. Using this model, we show that *Klebsiella pneumoniae* co- and super-infection enhances IAV infectivity but not when IAV was inoculated after bacterial infection. Enhancement was independent of cell lines, IAV strains, or *Klebsiella pneumoniae* strains but was not observed with other respiratory viruses like respiratory syncytial virus (RSV) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), nor other respiratory bacteria like *Pseudomonas aeruginosa* or *Acinetobacter baumanii*. Enhancement was associated with increased IAV RNA in the nucleus at 3 hours post infection, suggesting an early effect.

Here we will present mechanistic studies into how *Klebsiella pneumoniae* enhances IAV infectivity by considering interactions with the host cell innate immunity.

B437

Unravelling Species-Specific Differences in Mucosal Antiviral Immune Signalling in Protection From Emerging Respiratory Viral Infections

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Abstract

Emerging and re-emerging respiratory viruses like influenza A viruses, coronaviruses and paramyxoviruses represent major existential threats for humans and other animals. The outcome of infection is linked to critical interactions with host cells, of which the innate immune system forms a major barrier to infection. The type III interferon (IFN) lambdas are the dominant antiviral cytokine controlling antiviral immunity in the respiratory mucosa. Determining what aspects of mucosal IFNL immunity are conserved and what aspects vary may help us understand host-pathogen interactions, and develop novel interventions.

Here we present synthesis of experimental and computational approaches to dissect the genotypic and phenotypic diversity and evolution of IFNLs. *In silico* screening of vertebrate genomes revealed a diversity of IFNLs, with mammals harbouring two distinct lineages, termed herein IFNLA (IFNL4-like), and IFNLB (IFNL1-3-like). IFNLA and IFNLB showed distinct patterns of variation and copy number, with a notable expansion in placental mammals. Unlike IFNLA, IFNLB evolution was characterised by pervasive partial gene conversion. Mapping IFNL evolution onto the chromosomal locus identified instances of gene contraction and expansion. High-resolution mutation analysis guided by structural predictions identified instances of convergent evolution at receptor-binding interfaces, and in regions involved in protein folding and stability. Finally, functional experiments on recombinant IFNLs confirmed phenotypic effects of partial gene conversion on human IFNLs on the induced antiviral state in lung epithelial cells.

In conclusion, we present the first genotypic map of mammalian IFNLs, and begin to exploit this to chart the phenotypic landscape of IFNL variation at the species level.

B439

The role of non-structural protein 1 (NS1) in influenza A virus host adaptation

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Abstract

The non-structural protein 1 (NS1) of influenza A virus (IAV) is a key factor in antagonising the cell-autonomous immune response. Nuclear NS1 interferes with transcription termination by targeting CPSF30 and blocking host gene expression. NS1-CPSF30 binding is highly conserved in human-adapted strains and was positively selected for during the adaptation of the swine flu pandemic strain A/California/07/2009 (CA07) to the human host. While this indicates an evolutionary advantage of NS1-CPSF30 binding, it remains poorly understood how NS1-CPSF30 interaction contributes to IAV virulence and host shutoff in the human host.

Here we show that cellular infection with the original CA07 induces transcription termination defects in human lung epithelial cells, despite CA07 NS1's inability to block gene expression. Using cell-fractionation fand immunoprecipitation, we characterise nuclear and cytoplasmic interactors of human/swine NS1 of IAV-infected human/porcine lung epithelial cells.

Combined with assessment of global consequences of IAV-infection on host shutoff by nascent transcriptomics and translatomics, this project aims to contribute to our evolutionary and mechanistic understanding of NS1 which may improve risk assessment of IAVs.

B441

Uncovering SARS-CoV-2 mutational signature dynamics and variant effects using non-negative matrix factorisation and protein language models

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Abstract

During the SARS-CoV-2 pandemic, sequencing efforts proved vital in tracking the evolution and spread of this emerging virus. However, novel variants with altered viral properties were usually spread before their significance could be appreciated. The mutational processes that produce these variants occur in a complicated landscape of host factors. Mutations occur via replication errors or targeting by host antiviral molecules. These processes can be identified by the patterns of substitutions mutations (mutational signatures) left behind. Using non-negative matrix factorisation, we identify and quantify signature impact on the viral population over time. These mutations impact the structure of viral proteins and influence their phenotypic, including antigenic, properties. Protein language models have shown promise for assessing mutational impact. These models produce embedding representations that encode properties such as how a linear sequence is linked to protein structure. By embedding virus sequences of interest with every possible change (an in-silico deep mutational scan), we can derive embedding metrics to annotate these changes and estimate their effect. Using just a single reference sequence we can contextualise these scores and understand fundamental properties of the virus. Using the SARS-CoV-2 pandemic as a backdrop, we uncover the dynamics of mutational processes, estimate their contribution to the mutational landscape, and quantify the effects of spike protein-coding changes in the virus. We show how language models can be used to produce quick, actionable analyses that may prove useful in future pandemic scenarios. These approaches permit quantification of the cause and effect of viral mutations at a global scale.

BLOCK B

Session : Virus Workshop: Viruses: Molecular Machines to understand cellular processes

B442

The Universe of Transcription Factors Associated with ASFV RNA polymerase

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Abstract

African Swine Fever Virus (ASFV), a nucleo-cytoplasmic large DNA virus (NCLDV), causes a haemorrhagic fever in wild and domesticated pigs. Its rapid spread throughout Eurasia poses a threat to global food security and causes significant economic loss. The understudied gene expression and transcription mechanisms of ASFV present a crucial knowledge gap. ASFV, similarly to exclusively cytosolic replicating *Poxviridae* species, replicates predominantly within the cytosol and encodes its own RNA polymerase (RNAP), capping- and polyadenylation enzymes.

The ASFV core RNAP comprising 8 subunits has a molecular structure similar to the host RNAPII. Regulatory transcription factors associate with ASFV RNAP and modulate its function, including the temporal control of transcription. Sequence homology with known NCLDV factors can identify some of these factors, including D1133L and G1340L (D6 and A7 in Vaccinia virus respectively) that have been partially characterized and facilitate early transcription. Some late gene factors can be predicted with confidence, while other obvious candidates are missing entirely in ASFV; importantly the precise composition of ASFV RNAP complexes across the virus lifecycle remain unknown.

This project aims to characterise ASFV RNAP-associated factors in the virion and the infected cell during early and late infection. We will generate recombinant ASFV strains encoding affinity-tagged RNAP subunits, and subsequently purify RNAP-containing complexes from virions or infected cells and characterise their composition using mass spectrometry. We will produce recombinant versions of the identified factors, solve the structures of the complexes they form with the RNAP using cryo-EM, and dissect their function using *in vitro* transcription assays.

B443

In Silico and *In Vitro* Studies of African Swine Fever Virus Proteins to Aid the Generation of Viral Pseudotypes.

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Abstract

African swine fever (ASF) is a severe haemorrhagic contagious disease of Eurasian wild boar and domestic swine, which exhibits an exceptionally high mortality rate, usually 100% in acute infection. This is in part because the virus replicates to high titres before the host can mount an effective immune response. Moreover, there are no approved treatments or vaccines, although, several are in development. Therefore, the virus has been able to spread rapidly, causing the deaths of millions of pigs and massive economic losses; crippling the pig industry and threatening biodiversity.

The causative agent is a large, complex DNA virus that encodes for over 150 proteins and can be transmitted via multiple mechanisms (fomite, direct contact, soft-bodied ticks (sylvatic cycle)). Our understanding of ASFV is still limited in some areas, such as, which viral and cellular factors are involved in cell entry. We have sought to address this by attempting to generate pseudotyped viruses (PV) to study this stage of replication.

Based on published data and *in silico* analysis, utilising programs such as Alphafold, Foldseek and ChimeraX, a set of ASFV proteins potentially involved in cell entry were cloned. Their expression and incorporation onto the surface of PV was assessed by immunofluorescent microscopy, and their ability to infect susceptible cells by infection studies.

ASFV pseudotypes would greatly improve our ability to identify host and viral factors involved in cell entry. This information is important for researchers designing the much-needed treatments and vaccines to limit the impact of this deadly disease.

B444

An in-silico insight into the Egyptian fruit bat (Rousettus aegyptiacus) and the Australian black flying fox (Pteropus alecto) N6-methyladenosine (m6A) machineries: A step towards understanding the bat-viral emergence.

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Abstract

The N6-methyladenosine (m6A) is one of the most abundant RNA post-transcriptional modifications. Writers, erasers and readers are groups of cellular proteins that regulate this modification. Recently, the m6A has been proposed to modulate virus replication and innate immunity. *Pteropus alecto* (the Australian black flying fox) and *Rousettus aegyptiacus* (the Egyptian fruit bat) are two bats important in the transmission of zoonotic viruses yet remain neglected in the epi transcriptomics. In this study, we applied a range of in-silico tools to annotate and map differences and similarities of m6A machinery of *Pteropus alecto, Rousettus aegyptiacus* and *H. sapiens*. Initial annotation and phylograms of all m6A-related proteins of *P. alecto* and *R. aegyptiacus* clustered them within the mammalian cluster, grouped with other members of the Pteropodidae family; however, the tree of the m6A eraser, Fat mass and obesity-associated protein (FTO), placed the order Chiroptera (order of all bat species) as a separate clade. Additionally, the FTO recorded the lowest identity matrices in *P. alecto* and *R. aegyptiacus* when compared to the mammals and *H. sapiens* respectively. Syntenic gene analysis of the m6A loci of the two bats has identified, in 8 out of the 10 machineries, multiple flanking genes identical to those of the human loci. Protein sequence and structural comparisons have revealed the conservation of the two bats' writers while demonstrating mutational and structural variations for their erasers and readers when compared to humans. These studies will provide the foundation to underpin mechanisms of viruses-mediated exploitation of m6A machinery and its subsequent effect on the innate immunity of bats.

B445

Increasing genetic stability of recombinant rotaviruses through RNA sequence optimisation.

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Abstract

Rotaviruses are eleven-segmented RNA viruses infecting many animal species and humans globally. The segmented nature of the RNA genomes poses additional challenges for their assembly during replication. Moreover, genome segmentation creates multiple hurdles for generating genetically stable recombinant rotaviruses that can be utilised as new vaccine candidates. Here, we explore the impact of inter-segmental RNA interactions on rotaviruses' genetic stability through the use of bioinformatics to engineer mutants carrying a foreign RNA sequence. Non-cognate sequences including fluorescent proteins, as well as rotavirus protein NSP5 encoded by a different gene segment 11 were inserted into gene segment 5 via RNA structure optimisation to ensure their compatibility with other RNA segments within the rotavirus genome. Using this approach, we successfully rescued novel recombinant viruses, however, such sequence-optimised variants exhibited differences in their replication kinetics and genetic stability. These findings highlight the importance of RNA interactions in the genetic stability of recombinant rotaviruses.

B446

Characterization of the Contributions a Detoxified Stx-Prophage Makes to the Fitness of Its Bacterial Host

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Abstract

Stx-phages can horizontally transfer Shiga toxin-encoding genes between bacteria, and can enter the lysogenic cycle in their hosts. Stx-phages prophages can carry and introduce additional genetic material to the host cell and confer traits on their bacterial lysogen. This research focuses on identifying the function of prophage Φ24B towards the fitness of its host. Multiple approaches including RNA-seq, NanoString datasets, bioinformatics analyses, and motility tests were used. Results suggested prophage Φ24B contributes to the motility of its host, and a prophage gene *vb_24B_13c* can contribute to the motility of naïve cells even without the presence of other prophage genes. Results also suggested that temperature is a factor that affects the contributions of prophage to the fitness of its host. RNA-seq data, as validated by an amylase assay, showed that α-amylase activity is upregulated in the lysogen. Moreover, NanoString data revealed that overexpression of prophage repressor gene, *cI*, could potentially inhibit the expression of other prophage genes, including *vb_24B_13c*. This indicates that the lysogen's expression of *cI* is not high enough to repress these prophage genes. In conclusion, the expression of prophage *vb_24B_13c* can enhance the motility of its host cell by upregulating genes involved in flagellar synthesis and rotation, and it can inhibit the expression of some its downstream genes. Moreover, these results confirm that the prophage plays a significant role in reprogramming the metabolic function of the host, indicating a profound influence on the host's phenotype.

B447

Visualising the arenavirus RNA synthesis machinery in action

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Abstract

The *Arenaviridae* family of single-stranded RNA viruses contain species that are causative agents of haemorrhagic fever and death in humans. Despite this threat, there are no effective therapeutic or preventative measures for arenaviruses, resulting in the inclusion of Lassa arenavirus as a WHO priority pathogen. The arenavirus RNA genome is encapsulated within the viral nucleoprotein (NP), forming ribonucleoprotein (RNP) complexes in association with the viral RNA dependent RNA polymerase (RdRp). These RNPs are the arenavirus RNA synthesis machinery. The structure of these RNPs however is poorly characterised and there remain various fundamental questions regarding RNPs including how the RdRp is tethered to the RNP, and what exactly is responsible for RNP circularisation and flexibility.

Significant advances in cryo-electron microscopy (cryo-EM) have enhanced our structural understanding of viruses and viral RNPs. Here, we use the prototypical arenavirus lymphocytic choriomeningitis virus (LCMV) as a tool to investigate the structure of arenavirus RNPs, and identify fundamental details of RNA synthesis. Utilising a reverse-genetics system, we have successfully propagated and purified LCMV. In order to determine the native structure of RNPs, we have optimised methods to extract and purify RNPs directly from LCMV virions, resulting in successful disruption of LCMV virions to release the viral RNPs. Additional optimisation of the RNP purification method is required to further adapt these techniques for microscopy approaches. In turn, this will allow us to determine the native RNP structure using cryo-EM, and image the RNA synthesis process in action by atomic-force microscopy.

B448

Characterising viral degradation products during coronavirus infection

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Abstract

Coronaviruses are potential pandemic agents, as well as agents of common human and animal diseases. Understanding how these viruses replicate and interact with host cells is crucial for the development of new therapeutic approaches as part of future pandemic preparedness.During a typical viral infection, viral genes are expressed at very high levels, becoming some of the most abundant proteins in infected cells. Viral proteins are also subject to degradation in the same manner as host cell proteins, and as such degradation products can build up to noticeable levels. Some degradation products are stable and can include intact functional domains, raising questions as to whether such degradation products play discrete roles during infection and as such can potentially be targeted both for diagnostic and therapeutic purposes. This project focuses on mapping cleavage sites of SARS-CoV-2 and coronavirus proteins that do not correspond to known viral protease activity using N-terminomics.Using novel mass spectrometry approaches, these products can be quantified and analysed with a view to correlating observed data with pathogen functionality. Here we present new as well as re-analysed LC-MS/MS data on coronaviridae, identifying degradation products with the capability to have discrete functions and subcellular localization. These degradation products may represent new targets for antivirals and variation in their abundance may explain differences in the pathogenicity of viral variants.

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