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A001

PROBIOTIC CAPABILITY OF *Bacillus* spp. ISOLATED FROM "IRU" – A FERMENTED AFRICAN LOCUST BEAN (*Parkia biglobosa*)

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Abstract

Bacillus species have diverse applications in agriculture, enzyme production and medicine to produce vaccines and probiotics. Currently, little is known about the probiotic potential of *Bacillus* from fermented condiments of African origin. This work aimed at isolating *Bacillus* spp. with probiotic potential from fermented African locust bean – *iru*. *Iru* samples were obtained from a local market in Oyo, Nigeria. The samples were pretreated by boiling to eliminate vegetative cells and inoculated using the pour plate technique. Isolates were identified; their antibiotic susceptibility profile and antimicrobial activity against known pathogens (*Listeria monocytogenes, Salmonella enterica* and *Staphylococcus aureus*) were determined. Safety and technological properties (cellulase, protease, lipase, and amylase activities) of the isolates were also determined. Twenty spore-forming, Gram-positive with rod shape were isolated and identified as *Bacillus subtilis* (8), *Bacillus licheniformis* (4), *Bacillus pumilus* (3), *Bacillus polymyxa* (1), *Bacillus licheniformis* (1), *Bacillus alvei* (1), *Bacillus badius* (1) and *Bacillus* sp. (1). All isolates were susceptible to levofloxacin, ciprofloxacin, ofloxacin, gentamycin and azithromycin while 16 (80%) were resistant to cefotaxime. Only 11(55%) isolates inhibited the growth of *Listeria monocytogenes,* with no inhibition recorded against other pathogens. The 11 isolates synthesized at least one enzyme, with *B. subtilis* PA1 and *B. alvei* PB5 producing the four enzymes determined. γ-haemolysis was exhibited in 90% of the isolates while 10% exhibited α-haemolysis. *Bacillus subtilis* PA1, *Bacillus subtilis* PA6 and *Bacillus licheniformis* PA5 survived high acid, bile and; simulated acid and bile. *Bacillus subtilis* PA1, PA6 and *Bacillus licheniformis* PA5 from African fermented locust beans have excellent probiotic potentials.

A002

Strategies for deriving plasmids that are resistant to fertility inhibition

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Abstract

Conjugative plasmid vectors for carrying genes through populations such as the animal gut microbiome can be limited by Fertility Inhibition (FI) systems encoded by competitor plasmids already present in the population. The most common plasmids in gut Enterobactericeae belong to the broad F family. These encode the *pifC* gene that encodes an FI protein that targets the TraG protein of broad host range IncP plasmids which couples the conjugative replication complex to the Type IV Secretion System responsible for Mating Pair Formation. We cloned the pifC gene from F into an expression vector and confirmed that it can totally block IncP-1 plasmid transfer when up-regulated.

To specifically mutagenize the *traG* gene and isolate mutants resistant to fertility inhibition (FI^R) we deleted *traG* from IncP-1 plasmid pUB307 and complemented it with *traG* provided in a compatible vector that could be mobilized simultaneously with pUB307 transfer. The initial set up showed reduced sensitivity to FI suggesting that increased expression of *traG* could allow escape from inhibition. This prompted us to improve our mutagenesis strategy by decreasing the strength of the promoter upstream of the cloned *traG* to the point that FI is restored.

The observation also pointed to a second strategy in which a complete IncP-1 transfer gene-set could be restored with increased *traG* expression, potentially allowing FI^R without actually mutating *traG*.

Both strategies are currently being pursued and the progress to date will be presented.

A003

The bacteriome of microwaves

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Abstract

Many studies so far have described the use of microwaves to reduce the presence of microorganisms in foods. Microwave radiation extends food preservation by inactivating microorganisms, such as those from the genera *Escherichia*, *Streptococcus*, *Clostridium*, *Staphylococcus*, *Salmonella*, and *Listeria*. In this study, we analyse the microbiome of 30 microwaves from different environments: domestic use microwaves, shared-use domestic microwaves, and laboratory microwaves. The main aim is to determine if there is a characteristic microbiome of the microwave samples. Moreover, it is intended to describe if it is an anthropized microbiome and analyse whether other factors, such as the periodicity of cleaning or the power of use, affect the microbiome of microwaves.

A004

Understanding the bug that makes our drugs. A Multi-OMICs and genetic engineering approach to improve recombinant protein secretion in *E. coli*.

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Abstract

Recombinant protein production is an important tool used in research, synthetic biology and to produce biotherapeutics such as vaccines, antibody therapies and hormones. Production of therapeutic proteins is an industry estimated to be worth over $125 billion (1), with *E. coli* being one of the main expression systems used and around a third of therapeutic proteins produced in *E. coli* being secreted to the periplasm (eg. antibody fragments) (2).

However, production and secretion of large quantities of therapeutic proteins can be challenging as this often presents a burden on the cell and overwhelms protein production and secretion machinery.

In our study, a multi-omics approach combining transcriptomics and proteomics was used to determine cellular responses during secretion of a model therapeutic protein (scFv antibody fragment) under industrially relevant fermentation conditions. This increased our understanding of how *E. coli* responds to therapeutic protein secretion particularly with regards to stress responses, expression of genes involved in protein synthesis, folding, degradation and secretion and genes of unknown function.

This data was then used to direct engineering of strains and media for improved protein production and secretion. From the 15 candidate genes that were deleted or overexpressed, one that targeted membrane protein degradation improved yields of our model protein (scFv) under lab conditions. This strain is now being further tested to determine whether it improved yields of other therapeutic proteins and extended to industrially relevant conditions.

(1) https://www.mordorintelligence.com/industry-reports/recombinant-protein-market

(2) Castiñeiras et al., 2018, FEMS microbiology letters

A006

Ozone-Mediated Control of Food Spoilage and Food-Borne Pathogens

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Abstract

Post-harvest produce is subject to contamination by food spoilage and food-borne pathogens, and this is controlled in the food industry by surface sanitation steps. Disinfectants, which are currently used for this, can form toxic compounds, therefore ozone, which is a form of reactive oxygen species (ROS), may be a better alternative because it kills a broad range of micro-organisms without the formation of toxic residues. The use of ozone in the food industry has been limited because it is harmful to human health if inhaled, however in-pack ozone technology, developed at Glasgow University, mitigates the risks associated with ozone by containing the ozone within a sealed pack. The aim of the project is to investigate if in-pack ozone technology can be used to control plant and food-borne pathogens in the food industry. It was first important to determine how ozone impacted the native microbial community of post-harvest produce, therefore a large-scale 16S rRNA experiment was completed. Another aim is to investigate if the tolerance that bacteria may have evolved against another form of ROS, hydrogen peroxide (H_2O_2), is likely to aid the survival of pathogens during ozone treatment. This requires investigation because for pathogens to become established in plants or animals, they must be able to tolerate ROS (primarily H_2O_2), which is produced during plant defences and by phagocytes. Data has been generated which compares the transcriptional response of selected genes to both ozone and H_2O_2, to determine if model pathogens respond to each of these in a similar way.

A007

In-situ analysis of antimicrobial copper-titania surfaces for hospital infection control

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Abstract

Health care associated infections often result from the survival on surfaces of ESKAPE pathogens (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterococcus sp.*) To combat this, infection control interventions are required to break the chain of infection and reduce the rate of transmission.

The standard material used for hospital surfaces is stainless steel due to its corrosion resistance. As stainless steel does not naturally possess antimicrobial properties, ESKAPE pathogens can reside on these untreated surfaces in between periods of cleaning. Copper is effective as an antimicrobial coating but has not yet been widely adopted.

Our novel chemical vapour deposition (CVD) method deposits thin layers of copper and titanium onto stainless steel to create photocatalytic surfaces. We see potential in this method in manufacturing simple, cost-effective, durable, multi-mechanistic antimicrobial surfaces.

Our preliminary investigations show that copper/titanium surfaces demonstrate a marked decrease in viable *S. aureus* NCTC 8532 within 2 hours compared to untreated controls. This activity has not yet been quantified in a hospital environment with UV activation. Using culture- and 16S rRNA-based techniques, the current study will profile surface bacterial communities on copper/titanium hybrid surfaces *in-situ* within a variety of hospital environments over several months.

These investigations will test the feasibility of employing CVD to create sustainable antimicrobial surfaces to improve infection control in hospitals.

A008

Archaeal interactions with carbonate tufas

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Abstract

Lime kilns have operated for centuries to generate CaO for agricultural and industrial purposes. These inefficient processes generate significant quantities of waste, historically disposed into landfills. Percolating water through these deposits forms highly alkaline (pH > 12) and calcium-hydroxide dominated leachates which increases the subsequent precipitation atmospheric carbon dioxide as carbonates, referred commonly as tufa. Tufa formation further impacts local habitats by preventing gas exchange between the atmosphere and water source resulting in accelerated chemical degradation of plant material. The generation and subsequent fermentation of cellulose degradation products under anaerobic conditions provides substrates for methanogenesis. Recent studies suggest that carbonates may provide additional carbon sources for hydrogenotrophic methanogenesis.

An example of a lime kiln waste landfill is Brook Bottom Valley found within the Harpur Hill site, Derbyshire. Within the present study samples of tufa and the underlying soil from five sites were obtained. DNA extractions were conducted to determine the metagenomic profiles of the soil and tufa communities. Methanogenic enrichments were prepared from the soil (H2:CO_2headspace) and tufa (H2 only) to determine the communities ability to generate methane. After 14 days both the soil and tufa enrichment microcosms produced mM of methane and indicated the tufas potential of utilising carbonate as a carbon source for methanogenesis to occur. The soil enrichments displayed further methane production after 6 weeks within two different samples at pH 10 and 11. The data indicates that lime kiln landfill sites may be a source of atmospheric methane, future studies will consider the bioenergy potential of tufa.

A009

Insights into the diversity and function of Cyanobacterial mat structures in Antarctic lakes

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Abstract

Cyanobacteria have shaped biogeochemical ecosystems on Earth since their evolution approximately 2.5 billion years ago and are particularly successful at surviving in extreme environments. Under the right conditions complex cyanobacteria-dominated mats grow in high abundance and can form elaborate 3D structures, known as stromatolites, that are laminated through seasonal growth and incorporation of sediment. Perennially ice-covered Antarctic lakes provide low disturbance environments for microbial structures to dominate and are ideal systems to study the communities and environmental factors that shape them. These structures are of particular geological interest as they are modern day analogues of Archean stromatolites on early Earth.

While stromatolite communities have been studied previously, our knowledge of the genetic basis that shapes the formation of elaborate mat structures is still limited for perennially cold environments. In this study we used a combination of Illumina and Nanopore Oxford Technology shotgun metagenomic sequencing to assess the community structure and function of microbially-mediated structures in Lake Untersee, Antarctica. We evaluated the metabolic functional potential that supports energy and nutrient cycling within the extremophile benthic microbial communities and explore pathways associated with structure morphogenesis. These results provide key insights into the microbes and processes that influence the formation of microbial structures, past and present.

A010

Characterisation of *Pseudomonas putida* KT2440 solvent tolerance systems for the sustainable production of monoaromatics for the plastics industry

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Abstract

The plastics industry is reliant on petrochemical feedstocks for the synthesis of large-volume commodity chemicals. The petrochemically derived monoaromatic hydrocarbon styrene is used in the production of a number of polymers of industrial relevance including polystyrene and styrene-acrylonitrile resin. Styrene is produced from ethylbenzene via catalytic dehydrogenation, with ethylbenzene produced from the elementary petrochemical benzene. Both ethylbenzene and styrene are highly toxic to bacteria, limiting the choice of chassis organism: *Pseudomonas putida* KT2440 has recently drawn interest for the sustainable, biocatalytic production of large-volume commodity chemicals due to its metabolic flexibility, tolerance to oxidative stress and its amenability to genetic modification. *P. putida* crucially, is also naturally styrene/ethylbenzene tolerant, in part due to its suite of efflux systems making it ideally suited as a bacterial host for the sustainable production of ethylbenzene or styrene. Despite the structural similarity of ethylbenzene and styrene, we have found that *P. putida* is substantially more tolerant of ethylbenzene than styrene. Through a combination of transcriptomics and transposon-directed insertion site sequencing (TraDIS) we have identified a number of systems which are both highly upregulated and essential in tolerating both solvents. Despite their near identical structures, we have identified distinct transcriptional responses to ethylbenzene and styrene, with a number of different efflux systems implicated in response to both compounds. We have generated single and combinatorial deletions of these efflux systems in *P. putida* to establish their relative contributions to ethylbenzene and styrene tolerance in order to identify candidate systems for overexpression and improvement of tolerance.

A011

Methylation silent plasmid vectors for the genetic manipulation of industrially important Streptomyces species

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Abstract

Around two-thirds of all antibiotics currently in use are produced as specialised metabolites by Actinobacteria and over 80% of these are produced by the genus *Streptomyces*. Integrating plasmids are widely used in *Streptomyces,* exploiting bacteriophage-derived integrases to insert into specific sites in the genome of *Streptomyces spp.* They are important broad-host range genetic tools that are widely used for the complementation of mutants and the introduction of heterologous DNA into strains. One unusual feature of many *Streptomyces spp.* is the presence of a strict methyl-specific restriction enzyme system which efficiently degrades foreign methylated DNA. Therefore, conjugation of plasmids into many *Streptomyces spp.* is currently performed using *Escherichia coli* ET12567: a strain which lacks the *dam* and *dcm* methylation systems. This is problematic as *E. coli* ET12567 is slow growing, inefficiently transformed, and mutagenic so must be transformed with minimal delay before conjugation and is not appropriate for long term plasmid storage.

To overcome this problem, we have created a pair of *Streptomyces* integrating plasmid vectors based on pSET152 and pMS82 in which most Dam/Dcm recognition sequences have been inactivated by mutation. Initial testing showed our methylation silent version of pSET152 can be conjugated by DH5α directly into the model strain *Streptomyces coelicolor* with high efficiency. An organism which could not be efficiently conjugated with methylated pSET152.

We are hopeful that these two plasmids will allow easier introduction of DNA into the genomes of industrially relevant, antibiotic-producing *Streptomyces* and hence, simplify future research.

A014

Genomic and Phenotypic Characterisation of Two Myoviruses with Broad Lytic Activity Against Clinical and Veterinary Isolates of *Klebsiella* spp. and *Raoultella* spp.

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Abstract

Several species belonging to the genus *Klebsiella,* and the closely related genus *Raoultella*, are recognised as established and emerging human and animal pathogens. Increasing levels of antibiotic resistance, especially within the genus *Klebsiella*, can make treatment of infections difficult. Therefore, it is important that alternative treatment modalities are investigated. Bacteriophages (phages) are one such option.

Phages vB_KmiM-2Di and vB_KmiM-4Dii were isolated on strains of multidrug-resistant *Klebsiella michiganensis* and had broad lytic activity against clinical and veterinary isolates belonging to the genera *Klebsiella* and *Raoultella*. Greater than 85 % of the 100 *Klebsiella* and *Raoultella* spp. tested were lysed by either vB_KmiM-2Di or vB_KmiM-4Dii. Electron microscopy identified both phage had morphology typical of the *Myovoiridae.* They had genome sizes of 177,200 bp (275 genes) and 174,857 bp (271 genes), respectively. ViPTree and PhageClouds analyses showed both phages belonged to the genus *Slopekvirus*. Pangenome analysis of GenBank and metagenome-assembled *Slopekvirus* genomes (n=24) revealed a core genome of 155 genes, representing 57 % of all genes encoded in each genome. The pangenome (425 genes in total) was open. Despite a high level of sequence identity (95.25 %; VIRIDIC) across their genomes, differences in host range were observed between vB_KmiM-2Di and vB_KmiM-4Dii. We hypothesise this discordance may be due to differences in homing endonucleases encoded by these phages which, according to our phylogenetic analysis, appear to be divergent across the genus and contribute to the accessory genome.

In summary, due to their broad host range, these phages show promise as potential therapeutics.

A015

Testing the sit-and-wait hypothesis for the evolution of virulence in Streptococcus suis

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Abstract

Streptococcus suis is a commensal of the upper respiratory tract of pigs which can also cause opportunistic respiratory and systemic infections, as well as zoonotic disease in humans. In theory, pathogenicity should represent an evolutionary dead end for *S. suis,* but phylogenetic trees exhibit significant clusters of pathogenic isolates and diversification within pathogenic lineages. This suggests there are fitness benefits associated with pathogenicity resulting in its maintenance within *S. suis,* but these are not currently understood.

One prominent hypothesis of virulence evolution is the 'sit-and-wait hypothesis', which predicts a positive correlation between virulence and persistence in the outside-host environment. The hypothesis predicts that better environmental survival would allow increased indirect transmission via fomites, reducing dependence on host activity for transmission and thus permitting the evolution of increased virulence.

We used a comparative survival assay to empirically test this hypothesis in *S. suis* within a laboratory environment. We assessed durability on environmental surfaces by characterising the desiccation tolerance of 74 strains of *S. suis*, sampling at regular intervals over a period of approximately four weeks following desiccation to quantify the number of remaining viable bacteria.

We found that pathogenic strains died out significantly later than commensal strains at the p=0.05 significance level, suggesting that pathogenic isolates exhibit a greater average desiccation tolerance. Our results, which will be discussed further, contribute to our understanding of the evolution of pathogenicity in *S. suis* by providing empirical evidence in favour of the sit-and-wait hypothesis*,* which could inform efforts to control and prevent disease outbreaks.

A016

Climate Change, *Vibrio vulnificus* Risk and Human Health in North America

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Abstract

Vibrio vulnificus is an opportunistic bacterial pathogen, which occurs in warm low-salinity waters. *V. vulnificus* abundance in the natural environment mirrors ambient environmental temperatures, and as such, these bacteria are considered a tangible microbial barometer of climate change.

Frequency of infections are low, but impacts are high with mortality rates approaching 20% in humans. In spite of studies noting increasing bacterial concentrations in seawater, systematic assessments of changing disease patterns or projections of climate change impacts are rare.

V. vulnificus case data were obtained from the US Cholera and Other *Vibrio* Illness Surveillance (COVIS) database. An ecological niche modelling approach and CMIP6 data are used to predict the future distribution of *V. vulnificus* risk under different Shared Socioeconomic Pathways (SSPs) of climate change.

Here we show that in Eastern North America, the incidence of *V. vulnificus* wound infections has increased 8-fold between 1988 and 2018; the northern extent of cases has increased annually by 48 km during the same 30-year period. By 2090, we predict that *V. vulnificus* infections may expand from their current limit (~Pennsylvania, 39.98°N) to New York (40.71°N) under SSP126, a low emissions scenario, and to every Eastern Coastal US State under SSP370, a scenario of “regional rivalry”. These changes lead to an increase in the population at risk from 65 million (2007-2018) to 124 million and 88 million by 2090 under SSP126 and SSP370, respectively. This study highlights the importance of *V. vulnificus* as a key indicator of climate change.

A017

Glyphosate and Triticonazole Do Not Impact The Vectoral Capacity of *Aedes aegypti* for Zika Virus

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Abstract

Identifying susceptibility of mosquitoes to arboviruses is important for vector control and determining risk factors particularly with increased spread of these diseases. Laboratory studies are the gold standard for these studies, but mosquitoes are raised in clean environments that do not mimic a more complex environment. We investigated the effect of agricultural chemicals on vector competence. We identified a refractory strain of *Aedes aegypti* to Zika virus (ZIKV) infections. As a preliminary experiment, we aimed to identify if exposure to Glyphosate (herbicide) or Triticonazole (fungicide), both common agricultural chemicals that are found in run-off water and used in gardens, resulted in changes in vector competence. Cohorts of mosquitoes were treated with Triticonazole or Glyphosate, and then blood fed ZIKV post exposure to these chemicals. Whole mosquitoes were pooled into 10, triturated, RNA extracted and subjected to qPCR to determine presence of ZIKV. The results indicate that 37.5 and 25% of triticonazole and Glyphosate treated mosquitoes were infected with ZIKV respectively. Additional studies did not show any dramatic change in immune gene responses to exposure to these chemicals, suggesting that the presence of these chemicals may interfere with the virus replication in the midgut. Further work is required to identify if the microbiome plays a role in the change in vector competence, as well as identifying any mechanism that might be at play with virus replication.

A019

Engineering cyanobacteria as whole-cell biocatalysts for the light-driven production of the steroid drug 15β-hydroxytestosterone

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Abstract

The selective hydroxylation of steroid drugs by conventional chemistry is a complex reaction with a high environmental impact. The use of photoautotrophic microorganisms expressing heterologous monooxygenases could overcome this problem by fueling the reaction with electrons and O_2 derived from the light-dependent oxidation of water, occurring during photosynthesis. Here, the light-driven selective hydroxylation of testosterone into 15β-hydroxytestosterone was achieved using whole-cells of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 expressing the heterologous cytochrome P450 monooxygenase, CYP110D1. Additionally, the reaction conditions including cell density, aeration, and substrate concentration were optimized, leading to a maximum specific activity of 1 U g_{CDW}^{-1}. This value is about 2-fold higher than the one achieved using the model heterotrophic bacterium, *E. coli*, in which was necessary to express not only CYP110D1 but also its electron carrier proteins, and to add glucose as a sacrificial electron donor. Altogether, the results obtained here demonstrate the efficiency and sustainability of our engineered *Synechocystis* chassis for the oxidative biotransformation of a steroid drug.

A021

Investigation of the impact of food preservatives on avian gut microbiome and their role in driving zoonotic disease

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Abstract

The rapid emergence of antibiotic resistant bacteria is a major issue worldwide. The excessive use of antibiotics in agriculture is routinely described as a major contributor to bacterial resistance. Globally, antibiotics are widely used as growth supplements in livestock. This has led to concerns regarding human-use antibiotic use in food and food-producing animals. Lately, organic acids such as propionic acid (PA) and formic acid (FA) have been increasingly used as alternative antimicrobials or preservatives in place of antibiotics.

Recently, we have shown that exposure of a Crohn's Disease associated bacterial pathotype, Adherent-invasive Escherichia coli (AIEC), to PA significantly altered its phenotype resulting in increased adhesion and invasion of epithelial cells and increased persistence through biofilm formation (Ormsby et al.,2020). AIEC are both evolutionarily and phylogenetically related to avian pathogenic Escherichia coli (APEC). It's still unclear what makes APEC strains virulent in avian which remains a major issue in the poultry industry. Indeed, the widespread use of organic acids as growth supplements and antimicrobial in poultry industry, and the continuous exposure of microbes to organic acids is rising a concern due to the ability of organic acids to alter the bacterial pathotype. Here we investigated whether the increasing use of PA and FA in the poultry industry can lead to APEC strains that are increasingly virulent towards human cells with a potential for increased horizontal transmission.

A022

Light-driven cyanobacterial biotransformations are limited by the intracellular electron supply

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Abstract

Cyanobacteria are photosynthetic prokaryotes, currently under high attention for their abilities to capture solar energy and the greenhouse gas CO_2 for the production of high-value products. Recently, these have also been applied as host for photobiocatalytic reactions. Biocatalytic processes driven by photocatalytic reactions have emerged as an interface between renewable energy and chemical synthesis. Photobiocatalysis brings together photocatalysis and biocatalysis – two of the most research-intensive fields of catalysis.

Whole cell photo-biotransformations in cyanobacteria allow to exploit photosynthetic electrons for cofactor recycling saving organic auxiliary co-substrates. Oxidoreductases represent a strong electron drain, which allows to study how much of the photosynthetic electron transport chain can be deviated towards heterologous biotechnological processes, ranging from the production of high-value products to bulk chemicals and even biofuels.

With the example of a biocatalytic C=C bond reduction in engineered cells of *Synechocystis* sp. PCC 6803 overexpressing the gene of a bacterial ene-reductase, we showed that the reaction rate can be further increased by addition of glucose. The activating effect was observed at different cell densities and was independent from the light intensity during the cultivation and the choice of the substrate. The enhanced YqjM production rate in the presence of glucose is not caused by an increase in the photosynthetic electron transport rate. The results show that under photoautotrophic conditions, the ene-reductase is not NADPH saturated and has a higher capacity for substrate conversion, which might in the future be exploited for a deviation of the photosynthetic electron transfer chain towards heterologous biotransformations.

A023

Microbiota development, probiotic potential and competition with foodborne pathogens revealed by RNA-based long-amplicon sequencing during the fermentation of water kefir and kombucha

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Abstract

Fermented beverages such as water kefir (WK) and kombucha (K) have increased in popularity recently and have become known for their health benefits. This study mimicked the artisanal production of these beverages using a starter culture of grains (WK) or symbiotic culture of bacteria and yeast (SCOBY) (K). The first fermentation occurred at 25°C for 48 hours followed by a second fermentation at 25°C for 72 hours by adding ginger (~1g), before storage at 4°C for 40 days. Samples were taken at fixed points to identify and monitor the microbial community. Additional batches of WK and K were inoculated with a cocktail of pathogens and spoilage microorganisms (*S. aureus*, *S.* Typhimurium, *S*. Agona, STEC, *Yersinia* spp.) to observe their interactions in an environment with complex microbial communities. DNA extracted from the fermented beverages at different time-points were sequenced using a 16S rRNA long-read nanopore approach. Chao1 and Simpson indices showed small fluctuations in community composition. Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria were the dominant bacterial phyla. Both fermented beverages had distinguished microbial compositions, including lactic acid (*Liquorilactobacillus, Lactobacillus, Limosilactobacillus*) and acetic acid bacteria (*Gluconobacter*, *Komagataeibacter*) in K; while the community in the WK was more diverse, including prominent probiotic species (*Lactiplantibacillus*, *Lentilactobacillus Lacticaseibacillus* and *Leuconostoc)*, indicating the potential health benefits of this beverage. 16S rRNA of foodborne pathogens were detected in both the experimentally-spiked beverages at day 1 only, highlighting the importance of the starting cultures in guaranteeing the safety of these artisanal fermentations by competing microorganisms.

A024

Scottish hospitals and communities have distinct waste water resistomes: a cross-sectional metagenomics study of 8 waste water networks in Scotland

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Abstract

Background: Applying metagenomics to sewage samples is a convenient and informative way to investigate the human resistome. We applied metagenomics to sewage samples from multiple Scottish hospitals, community sites, and their connected waste water treatment plants (WWTPs). We aimed to assess the links between hospital and community resistome and prescriptions using paired samples, statistical and machine learning methods.

Methods: We sampled from 8 WWTPs (4 with a district hospital in their catchment area, and 4 without), and at an upstream site for each WWTP (either a hospital or a community site). We obtained antibiotic prescriptions dispensed to the communities and hospitals sampled. Samples were sequenced using Illumina NovaSeq and mapped to the ResFinder database using KMA. We used zero-inflated multi-level linear regression, cluster analysis, and source-attribution random forest methods to compare resistance in hospitals, communities, and prescription rates.

Results: Hospital sewage resistomes were more similar to each other than to community or WWTP resistomes. WWTP resistomes were unlikely to have hospitals attributed as their source (14% with and 12% without hospitals in their catchments), and WWTP resistance gene abundance was not affected by hospitals (regression coefficient 0.3, -1.0 – 1.5). There was no overall correlation between prescription rates and resistance levels, although vancomycin resistance and prescriptions in the community positively correlated (regression coefficient 14000, 6500 – 23000).

Conclusion: We provide evidence that a) WWTP influent is a useful indicator of the community resistome; b) hospital and community/WWTP sewage resistomes are distinct; and c) hospital resistome are similar, suggesting common drivers.

A025

Effect of Miswak Chewing Sticks on Oral Helicobacter Pylori under both fasting and non-fasting conditions – A crossover randomized clinical trial

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Abstract

Objectives: The chewing stick or “miswak” has demonstrated growth-inhibitory effects on several oral micro-organisms including *Helicobacter pylori (H. pylori)*. This study explores the effect of miswak chewing sticks on oral *H. pylori* among fasting and non-fasting subjects.

Materials and Methods: The study was a single center, single blind, randomized and cross-over study. This study included 20 subjects who were on 12-hour fasting (fasting group) and 20 subjects who were not fasting (non-fasting group). At baseline (T0), both the fasting and the non-fasting groups were sub-divided randomly into Group-1 and Group-2 (n=10 each). Group-1 subjects were instructed to use both toothbrush and miswak for two weeks (T1) and only toothbrush (TB) for the next two weeks (T2); and, Group-2 subjects were asked to use only TB during T1 and TB+M during T2. Clinical periodontal parameters were recorded at T0. Plaque and salivary samples were collected at T0, T1 and T2 and quantification of *H. pylori* from the samples were performed by real-time polymerase chain reaction (qPCR).

Results: A statistically significant correlation was observed between the quantity of *H. pylori* in plaque and clinical attachment loss ($p<0.001$) and probing depth ($p=0.001$) at T0. For non-fasting subjects, there was statistically significant reduction in the *H. pylori* counts in dental plaque samples for the subject using toothbrush and miswak as compared with those using only toothbrush ($p<0.05$).

Conclusions: Non-fasting subjects showed significant reduction in the *H. pylori* counts in dental plaque by complementing miswak chewing sticks with toothbrushing.

A026

Taxa-specific differences in the bacterial content of hand-washing sink drains located in clinical and non-clinical areas of a UK hospital

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Abstract

Recent years has seen increasing recognition that hand-washing sinks and wastewater drains harbour antibiotic resistant organisms, including carbapenemase-producing members of the Enterobacterales (CPE). However, most studies reporting CPE fail to consider the wider ecological niche within which these organisms are present. Herein, we examined water- and surface-associated communities sampled from hand-washing sink drains located in clinical and non-clinical areas of a UK hospital with a high prevalence of CPE colonisation to gain a fuller understanding of their composition and how drain trap replacement affects colonisation of this niche. Both selective culture-based methods and 16S rRNA sequencing were used to determine the bacterial composition of samples taken from 7 sinks in 2018 and 5 sinks in 2019. Culturable populations of heterotrophs and coliforms in drain water were relatively stable over time, while CPE populations were found to vary over 5 orders of magnitude across sinks and years. 16S rRNA sequencing revealed both water and surface-associated communities were dominated by Proteobacteria, contained equivalent (high) levels of diversity and a similar compositional structure. Linear discriminant analysis (LEfSe) however, revealed that samples taken from sink drains in clinical areas contained significant more *Burkholderiaceae* and Melainabacteria, and significantly less *Pseudomonaceae* than those from non-clinical areas (e.g. in toilets, kitchens). Drain trap replacement did not prevent the re-establishment of CPE nor lead to significant changes in composition or diversity. These findings highlight the high taxonomic diversity found within sinks and suggest that the abundance of specific taxa is impacted by location, and who uses them.

A027

Modulation effect of different concentrations of MWCNTs and nanometric ZnO on pyocyanin production by *P. aeruginosa*

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Abstract

Pyocyanin is a pigment recognised as a virulence factor of *Pseudomonas aeruginosa.* However, this metabolite presents various potential applications in, e.g., energy production in microbial fuel cells, fabrication of sensors and OLED screens, or antitumour therapy. Recently, it has been found that nanomaterials may influence pyocyanin yield which opens a new research route for metabolic engineering. Following the possible toxicity of nanomaterials and medical consequences of pyocyanin production, most research so far focused on reducing pyocyanin production. The enhancement of the pigment excretion was noted by a few groups although their findings need to be developed in greater detail. Therefore, the goal of the presented research was to assess the influence of the temperature and a wide range of concentrations of multi-walled carbon nanotubes and nanometric zinc oxide on pyocyanin production by *P. aeruginosa.*

The parameters recorded in the study included the optical density of the culture, as well as its fluorescence, biofilm biomass and viability, and pyocyanin production. The results indicating the influence of the culture temperature and nanomaterials used in different concentrations were subjected to statistical analysis following the Design of Experiment (DoE) approach. Moreover, scanning electron microscopy was used to assess the influence of nanomaterials on the cell morphology and the biofilm structure.

The obtained results showed that pyocyanin production depended not only on the concentration of the nanomaterial but also on its type. The outcome confirms the previous findings and strengthens this novel approach for obtaining high concentrations of pyocyanin through the optimization of bioprocess conditions.

A028

Universal Culture and Microscopic Imaging of Soil Microorganisms Using Transparent Soil and Soil Extract Medium

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Abstract

Imaging soil organisms in their natural habitat can be challenging to the microscopist due to the opacity and complexity of soil. To this end, much of what we know about soil microorganisms has been through the use of lab-based culture – agar and liquid media – that do not accurately represent the environment from which they were isolated. In the lab, Aspergillus niger typically grow as radial macrocolonies on agar plates. These structured macrocolonies are not typically seen in nature and may not physically or chemically reveal how Aspergillus grows or behaves in soil. However, with the advent of nafion based transparent soils, the soil environment has been more accessible to the microscopist – allowing for imaging of plant root and microbiological processes. Here, using confocal microscopy, we present four-dimensional data of foraging behaviour displayed by Aspergillus niger, using mycelia to span the gap between transparent soil particles in search of nutrients. Furthermore, we present a novel method of preparing transparent soil with real soil nutrients with the aim to further mimic the soil environment. Using this approach, we overcome previous limitations of the system that disallowed universal culture of soil microorganisms. Our method allows for culture of various bacteria and fungi using an identical setup, unlike previous methods requiring strain-dependent culture conditions.

A029

Commercial Kit Comparison for the Extraction of Long-read DNA from Plastic-degrading Microbial Communities

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Abstract

Metagenomic analysis is an important tool used for the exploration of novel plastic-degrading microorganisms. Whilst the development of next-generation sequencing (NGS), particularly long-read approaches such as Oxford Nanopore Technologies (ONT), have greatly advanced the study of metagenomics, recovery of high-quality, long-read genetic material from microbial populations remains challenging. Though chemical lysis retains DNA integrity, certain microbial species (e.g., Gram-positive bacteria) often require vigorous lysing methods such as bead-beating. These result in greater shearing of DNA, producing more fragmented reads, which require further bioinformatic processing and assembly. There is a trade-off between sufficient lysis to isolate DNA for sequencing and maintaining integrity of genetic material for long-reads. For microbial populations containing unknown species, it is necessary to use approaches that satisfy both requirements to ensure that extraction biases are minimised, but high-quality DNA is obtained.

To investigate which kit best achieves this trade-off, a ZymoBIOMICS Microbial Community Standard was used as a proxy to evaluate DNA extraction efficiencies from three commercially available kits. These represent different methodologies, including magnetic bead purification (Quick DNA/RNA MagBead Kit, Zymo Research), column-based membrane purification (AllPrep DNA/RNA Mini Kit, Qiagen) and alcohol precipitation (RNeasy PowerSoil DNA Elution kit, Qiagen). Using each kit, DNA was extracted, 16S rRNA amplified and sequenced using the ONT GridION, alongside whole DNA.

Results suggested that each kit showed distinct biases in extraction efficiency, with differing effects on DNA integrity, signfiying one kit in particular indicating high suitability for environmental sampling of novel plastic degrading microorganisms.

A030

The impact of hydrodynamic conditions and nanomaterials on the rhamnolipids production process

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Abstract

Rhamnolipids are biosurfactants composed of rhamnose, as a sugar moiety, linked to β-hydroxylated fatty acid chains. *Pseudomonas aeruginosa* is one of the main producers of rhamnolipids. These biosurfactants can be used in medical, agriculture, food, and petrochemical industries. Due to their environmental friendliness, a wide range of properties, and biocompatibility, further optimization of rhamnolipids production is desired. Therefore, many research teams use different approaches to intensify the process, e.g., medium optimization, bioengineering. Recent findings on the potential stimulation of physiological features in bacteria caused by nanomaterials or electromagnetic fields opened novel possibilities to obtain a higher yield of this metabolite. The aim of this study was to examine the influence of hydrodynamic conditions and different concentrations of nanomaterials (graphene oxide and magnetite) on the rhamnolipid production by *Pseudomonas aeruginosa.*

The effects of hydrodynamic conditions and nanomaterials on bacterial culture were evaluated using optical density measurements. Rhamnolipid concentration was performed with methylene blue assay. In addition, the biosurfactant production were tested using the emulsification index and drop-collapsing test.

The obtained results show that both hydrodynamic conditions and different nanomaterials concentration can influence the final rhamnolipid concentration. The production of rhamnolipids can be increased by specific hydrodynamic conditions and nanomaterial concentration.

A031

Identification of toxigenic *Vibrio parahaemolyticus* at Irish shellfish production sites.

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Abstract

Intro: *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* are marine pathogens that cause gastroenteritis in humans after the consumption of infected shellfish. Oysters are significant vectors for *Vibrio* disease. This project investigates the occurrence and distribution of pathogenic and toxigenic *Vibrio* in farmed Irish shellfish and the association with seasonal fluctuations in temperature and salinity to determine the potential risk to shellfish production and human health

Methods: Developed a programme of shellfish harvesting and environmental monitoring throughout a 12-month cycle at three shellfish farming locations on the West coast of Ireland. Shellfish, water and sediment samples were analyzed for *Vibrio* using culture and genetic approaches. *Vibrio* isolates possessing toxigenic virulence genes associated with disease in humans were identified via PCR.

Results: Our *Vibrio* monitoring programme started in November 2020. So far we have detected *V. parahaemolyticus* at shellfish farm locations, in sediment, oysters and water samples. Neither *V. cholerae* nor *V. vulnificus* were detected. PCR analysis identified the presence of Type Three Secretion System Two (TTSS2) toxigenic genes in samples from two of our sampling locations, including both alpha and beta TTSS2 genes. Suggesting that *V. parahaemolyticus* isolate serotypes differ based on sampling location.

Conclusions: *V. parahaemolyticus* bacterial communities differ based on geographical location and are present in Irish shellfish production areas. By identifying the risks posed by pathogenic and toxin producing Vibrio, measures will be proposed and put in place to minimise and overcome these threats to both the shellfish consumers and shellfish producers.

A032

High pH anoxic methanogen bioreactors- What processes are occurring and why can they survive?

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Abstract

Samples taken from former lime kiln, quarry, and steel slag sites were used to prepare bioreactors, studied in anoxic conditions at pH 10-11. Hydrogen consumption and methane production was monitored using headspace gas analysis using gas chromatography using Agilent GC6850.This identified the likely occurrence of hydrogenotrophic methanogenesis in these reactors. However, as the amount of methane being produced is not equal to the ratio of hydrogen beingc onsumed, this implies it is not the only pathway active within these bioreactors. Such as homoacetogenesis, syntrophic acetate oxidation, acetoclasic methanogenesis, or methylotrophic methanogenesis. The fact that these closed systems had survived for months with no new hydrogen being introduced, and that they produced methane as soon as new hydrogen entered the system, is further evidence for this. Current research does not explore methanogenesis at pH 10–11, or whether it is possible for such bacteria to use these pathways in highly alkaline conditions.

Additional VFA analysis of the reactors carried out using a HP GC6890 gas chromatographer identified fluctuation and variance in acetate concentrations within the bioreactors. This supports the theory that other pathways are occurring. The observation that acetate concentrations increased as hydrogen was introduced to the system suggests this could be acetoclastic methanogenesis or homoacetogenesis. The observation that dissolved inorganic carbon (determined using Aurora 1030 Wet Oxidation TOC Analyze) decreased as hydrogen was reintroduced further supports this idea.

A033

Selection for Antimicrobial Resistance by Aminoglycoside Plant Protection Products in Microbial Communities

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Abstract

Crop production utilises the application of plant protection products (PPPs) including antimicrobials in many countries, to crops and soils. Bacterial exposure to PPPs may result in the development and spread of antimicrobial resistance (AMR) genes between environmentally and clinically relevant bacteria. Concerns have been raised about elevated levels of antimicrobial resistant bacteria in environmental compartments receiving pharmaceutical waste, where antimicrobial drugs are found at concentrations of µg-mg/L. Despite this, the application of antimicrobials to crops and soils at >mg/L has received little attention. Selection pressures exerted by aminoglycoside PPPs and how they impact AMR will be addressed in this presentation.

Lowest observed effect concentrations for streptomycin (2mg/L), gentamicin (0.25mg/L) and kasugamycin (~0.78mg/L) were determined. Results informed the concentration range for seven day evolution experiments. QPCR for 16S rRNA and *intI1* (a marker for resistance) was performed on evolved communities to determine changes in *intI1* prevalence. Metagenomic sequencing was conducted and analysed using 'MetaPhlAn2' to determine changes in community structure. ARGs-OAP (version 2) was used to quantify relative abundance of resistance genes. At the class level, selection for aminoglycoside, fosmidomycin and sulfonamide resistance was observed at concentrations below application for streptomycin and gentamicin. Additionally, streptomycin selected for kasugamycin, quinolone and vancomycin resistance. Gentamicin also selected for β-lactam, MLS, tetracycline, trimethoprim, bacitracin and chloramphenicol resistance. In kasugamycin exposed communities, significant selection was only observed for quinolone resistance at application concentration.

Results provide information on the effects of antibiotic PPPs on microbial communities, contributing to understanding AMR from a “One Health” perspective.

A034

Characterising the diversity and antimicrobial resistance of Enterobacteriaceae as emerging key risk industrial contaminants

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Abstract

Enterobacteriaceae bacteria, well known to cause infection, are emerging as problematic Gram-negative contaminants of preserved (non-food) industrial products. The absence of routine genomic surveillance and characterisation of contaminants means that their species diversity and antimicrobial resistance is poorly understood. Using genome sequencing and phylogenomics we showed that a collection of 23 Enterobacteriaceae isolated from Home and Personal Care products or factories comprised of 10 species from 7 genera : *Pluralibacter, Enterobacter, Klebsiella, Leclercia, Citrobacter, Siccibacter* and *Kosakonia*. *P. gergoviae* was dominant within the collection (n =13), and industrial strains were closely related to clinical strains. 24 clinical and 14 environmental Enterobacteriaceae were incorporated into the collection, and the ability of the 61 strains to tolerate 10 preservatives (alcohols, organic acids, nitrogen compounds, chelators), and 18 antibiotics (Beta-lactams, cephalosporins, aminoglycosides, fluroquinolones, misc.), were determined by agar/broth dilution and disk diffusion assays respectively. Industrial isolates were significantly more tolerant ($p>0.05$) of organic acid preservatives (e.g. sodium benzoate, potassium sorbate) and benzalkonium chloride than Enterobacteriaceae from clinical or environmental sources. A taxonomic breakdown of elevated preservative tolerance revealed it was largely associated with *P. gergoviae* contaminants. Multidrug resistance was most prevalent in the clinical strains examined. Despite the presence of antibiotic resistance genes within their genomes the industrial Enterobacteriaceae demonstrated sensitivity to 13 of the 18 antibiotics: only 4 strains had resistance to more than one antibiotic. This research advances our understanding of the risks of Enterobacteriaceae contamination and facilitates the rational design of strategies to prevent it.

A035

Global scale comparisons of phosphorus metabolism in marine prokaryotes

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Abstract

Phosphorus (P) is an essential element for all life on Earth and its bioavailability to microbial species has a critical role in marine biogeochemistry and the functioning of marine ecosystems. Intertwined with the cycles of other major biogeochemical elements carbon (C) and nitrogen (N) P has a strong impact on the Earth's climate and productivity in the oceans[JC1] . Traditionally, phosphorus starvation was thought to regulate the expression of phosphorus metabolism genes, but recent data has shown this isn't the case for some pathways. Additionally, we have a poor understanding of the relative prevalence of different pathways. Therefore, we explored P metabolism genes using publicly available data compiled by Sunagawa *et al.*, (2015) who assembled high-quality reads into contigs assigning function using Clusters of Orthologous Groups (COGIDs) of 243 Tara Ocean metagenomic samples from 68 locations. This showed that although the alkaline phosphatases are traditionally thought to be the most important method for P acquisition, some phosphonate metabolism genes are equally important and become more important than the phosphatases with increasing ocean depth. Whilst P availability is tightly correlated to some P metabolism pathways such as polyphosphate, other pathways such as those for phosphonate catabolism are present in samples taken from locations that are not P limited. This suggests that some P metabolism genes are not regulated by P availability.

In this work we present a comprehensive dataset detailing the P metabolism pathways that are the most important for marine prokaryotes for P acquisition relative to P availability and ocean stratification.

A038

Natural product discovery for novel antibiotics from soil bacteria

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Abstract

The current spread of antimicrobial resistance represents one of the most critical threats to human health worldwide. Most classes of antibiotics currently in medical use were discovered from soil bacteria. However, most environmental bacterial species are uncultivable under laboratory conditions. This portion can still harbour a great diversity of antibiotics that is unexploited.

The aim of this project is to explore and develop unique sources of natural products by developing culture-dependent as well as culture-independent methods to study, cultivate and isolate acidobacteria from Antarctic soil. The focus is to investigate and isolate natural product biosynthetic gene clusters (BGCs) from strains, express these BGCs, then determine biological activities and chemical structures of the novel natural products.

The culture-dependent methods involved enriching soil microcosms using selective substrates and conditions, then isolating acidobacteria as well as other bacterial groups. A collection of 904 isolates have been obtained and high throughput screening of the isolates using 16S long amplicon sequencing was applied. Genome analysis of selected isolates will be achieved by linking short-read and long-read genome sequencing. The optimal enrichment conditions for the bloom of these indigenous acidobacteria will be compared through metagenomic analysis of the microcosms' microbiome to determine which set of conditions resulted in the most significant enrichment for amplifying acidobacteria in the population growing within the incubated soil.

A039

Potential of BONCAT to measure bacterial growth at a single-cell level

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Abstract

Bioorthogonal non-canonical amino acid tagging (BONCAT) has shown promise as a culture-independent method to measure bacterial *in situ* protein synthesis at a single-cell level. The rate of protein synthesis is intimately linked to the bacterium's growth rate, as proteins are essential building blocks for the growing cell. Bacterial growth rate is traditionally measured through optical growth curves, that are culture-dependent and can be challenging to perform for slow-growing bacteria. Furthermore, optical growth curves only measure growth at population-level, overlooking individual heterogeneity within the population. We performed some ground-laying experiments to assess whether BONCAT can be used to measure bacterial growth rate, using the *Escherichia coli* model strain DSM 103246. We used the methionine analogue L-azidohomoalanine (AHA) for the experiments, and measured the BONCAT fluorescence with the help of flow cytometry. By performing BONCAT incubations at different points of the strain's growth curve, we were able to show that the BONCAT signal was the strongest during early exponential phase, corresponding to the growth phase showing the fastest growth rate. In addition, by incubating exponentially growing *E. coli* at different temperatures, we found that the weakest BONCAT signal was observed at the lowest temperature. We conclude that BONCAT shows promise as a culture independent method to assess bacterial growth rate, however, systematic studies on different bacterial species are needed to determine the applicability of BONCAT for growth-rate determination in natural microorganisms.

A040

Abiotic Stress Interactions Restrict Bacterial Populations Ability to Alleviate Stress via the Formation of Spatial Refuges

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Abstract

Sea-level rise and droughts associated with climate change has accelerated the global salinisation of arable land. Increasing salt levels affects 24% of irrigated land worldwide, posing a threat for food-, medicinal- and industrial-crop-security, as well as social and economic issues.

Despite this threat, little is known about the ability of soil microbes to adapt to increasing salinity, and how these adaptations contribute to a population's persistence. This is surprising given the well documented importance of microbes for nutrient cycling and crop-protection, including protection from salinisation.

To investigate microbial evolution to increased salinity, and how adaptation is affected by abiotic conditions, we used a series of multifactorial evolution experiments to investigate the ability of the plant promoting bacterium *Pseudomonas fluorescens* to adapt to a high salt environment. Furthermore, we examined how the ability to adapt to high salt is impacted by prominent agricultural conditions: nutrient limitation (Caused by overuse of arable land, desiccation, and collapse of nutrient cycles), and agitation (e.g., tilling, unstable soil, and drainage).

Our results show that microbes can survive under elevated salinity levels, but agitated or nutrient limited conditions exacerbates the impact of salt stress, driving extinction. We identified the evolution of a biofilm phenotype which increases fitness under salt stress. This phenotype is supressed under low nutrient conditions, but expressed under high salt even if nutrients where low. We hypothesise that microbes can form biofilms that act as spatial refuges to survive high salt, however, this capability can be constrained by the abiotic environment.

A041

Comparative analysis of diversity and environmental niches of soil bacterial, archaeal, fungal and protist communities reveal niche divergences along environmental gradients in the Alps

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Abstract

Although widely used in ecology, comparative analyses of diversity and niche properties are still lacking for microorganisms, especially concerning niche variations. In this study, we identified important topoclimatic, edaphic, spatial and biotic drivers of the alpha and beta diversity of bacterial, archaeal, fungal and protist soil communities. Then, we calculated the niche breadth and position of each taxon along environmental gradients within all taxonomic groups, to determine how these vary within and between groups. Quantifying the niches of microbial taxa is necessary to then forecast how taxa and the communities they compose might respond to environmental changes. We found that edaphic properties were the most important drivers of both community diversity and composition for all microbial groups. Protists presented the largest niche breadths, followed by bacteria and archaea, with fungi displaying the smallest. Niche breadth generally decreased towards environmental extremes, especially along edaphic gradients, suggesting increased specialisation of microbial taxa in highly selective environments. Overall, we showed that microorganisms have well defined niches, as do macro-organisms, likely driving part of the observed spatial patterns of community variations. Assessing niche variation more widely in microbial ecology should open new perspectives, especially to tackle global change effects on microbes.

A042

Microbial colonisation of Arctic soils during snow melt

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Abstract

Arctic soils, as elsewhere, are subjected to microbial invasion, either from airborne, marine or animal sources. However, in winter, the snowpack isolates Arctic soils from outside sources, with the snow as the sole source of microorganisms. A successful colonisation depends on the alpha diversity, ability to survive and compete of both, the invading and resident community(Mallon *et al.*, 2015). This study monitored snow and soil microbial communities throughout snow melt to investigate the colonisation process of Arctic soils during melt. Results indicated that microbial colonisation likely occurred as all the primary conditions for a successful colonisation were fulfilled. As the colonising microorganisms originated from the snow system, subjected to similar conditions as the soil, the snow microbes were already adapted to the local environmental conditions. Furthermore, competition-related genes increased in snow samples as the snow melted. 100 potentially successful colonists were identified, demonstrating the deposition and growth of snow microorganisms in soils during melt. However, whether these potential colonists successfully colonised the soil long-term remains undetermined. Furthermore, no clear impact on the soil functional profile within the time frame of this study was observed, despite the expected influence of successful colonisation on the taxonomic and functional profile of the resident community.

A043

New carbohydrate-active enzymes for improved detergent sustainability

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Abstract

Many detergent formulations are currently formulated with carbohydrate-active enzymes (CAZymes) designed to improve cleaning by targeting a variety of distinct polysaccharide-based soil components. Current CAZymes used for this application include amylase, mannanase, pectate lyase, xanthan lyase, licheninase and cellulase. Recent analysis of residual polysaccharide soils left on clothing following washing suggests potential for other enzyme classes to be used to improve cleaning by targeting these polysaccharides. This could help pave the route for washing in more environmentally friendly colder and quicker washing conditions and improve the sustainability of detergents by replacing harsh petrochemicals. The aim of the project is to identify and produce novel CAZyme candidates designed to target residual polysaccharides, for example through the mining of metagenomes and by studying the secreted enzymes of microorganisms known to degrade the target polysaccharides, including Bacteroides strains. Work involves collaboration with partners at Newcastle University to study interactions between the enzymes and polysaccharides, and visits to Procter & Gamble's Newcastle Innovation Centre to evaluate the enzymes produced in washing performance tests. Results to date will be presented.

A044

Metagenome-assembled genomes from ancient Scărișoara Ice Cave provide details of bacterial evolution and climate conditions over the last 1500 years

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Abstract

Ice Caves are unusual habitats characterized by extreme conditions for life, such as constant dark, extreme cold, and oligotrophic conditions. The Scărișoara Ice Cave is one of the oldest and largest perennial underground ice-blocks in the world. The Ice block is formed via the annual freezing of a layer of water that trickles into the cave during the summer months, forming a shallow lake on top of the existing ice block. Over centuries of annual freezing, this process has resulted in sequential ice layers of variable thickness, separated by organic-rich sediment layers. This makes it a fascinating record of past climatic conditions. To investigate bacteria that can survive in this environment (past and current), metagenome-assembled-genomes (MAGs) were constructed from a co-assembly of shotgun data from seven sites within the cave ranging from present-day to approximately 1500 years old. The abundance of the MAGs at various sites was inferred via read mapping, and the MAGs were investigated for their biogeochemical cycling and metabolic potential. There were 121 high-quality MAGs assembled from 13 bacterial phyla and one archaeal phylum. The majority represent novel species. There were strong spatial patterns amongst the community members and mapping reads back to contigs enabled the resolution of distinct strains of closely related species. There were several biogeochemical pathways suggesting that anaerobic photoautotrophy is possible near the entrance to the cave, but chemolithotrophy is common at other sites. This data provides a fascinating insight into novel species, extremophilic lifestyle adaptations, and past climate and environmental conditions.

A045

Metagenome-assembled genomes provide insight into functional connections between bacterial communities from High Arctic glacier ecosystems

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Abstract

High Arctic glaciers house substantial microbial diversity and biomass capable of biogeochemical cycling of various nutrients and gases with direct implications for global climate change. Metagenome-assembled genomes (MAGs) typically represent the most abundant members of a community and can be analysed for genes and genome clusters that sustain ecosystem functioning and biogeochemical cycling. Whereas MAGs have found utility in describing some of the uncultured genomic diversity of terrestrial and marine habitats their application to Arctic environments and glacier habitats is limited. In this study, via co-assembly of soil, cryoconite, and seawater metagenomes, it was possible to reconstruct 74 high and medium-quality MAGs from the most abundant community members. The MAGs belonged to species detected in high abundance by numerous 16S rRNA gene surveys of the same sites. The MAGs represent 14 different phyla, and 72 MAGs represent novel species, although several are closely related to known psychrophilic species. The mean coverage of the MAGs and their distribution across different samples, tell us about their abundance and distribution in various environmental sites. There was a substantial core of cryoconite bacteria that spanned across two glaciers and persisted over two sampling seasons. Notably, six cyanobacterial MAGs were resolved, which appear to have strong habitat preference and possibly play vital roles in cryoconite and early soil formation. In addition, the presence and absence of several enzymes involved in the major biogeochemical cycles were described. The MAGs assembled from this environment provide important ecological information about this highly threatened environment.

A047

The dirty secret of Scottish beaches: human pathogens and sewage-associated plastic waste in the Forth Estuary

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Abstract

Sewage-associated plastic wastes, such as wet wipes and cotton bud sticks, are a legacy of sewage discharge to the environment. Such waste commonly washes up on beaches, and whilst this is unsightly, it is unclear whether this remains a public health risk by acting as a reservoir for human pathogens. In this study, sewage-associated plastic waste, and naturally occurring substrates including seaweed and sand, were collected from ten beaches along the Forth Estuary (Scotland, UK) and analysed using selective media to detect *Vibrio* spp., and the faecal indicator organisms (FIOs) *Escherichia coli* and intestinal enterococci. Multiplex PCR was used to further identify isolates of *Vibrio,* including *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus,* that may present a greater risk to human health. The sensitivity and resistance to a range of antibiotics was also determined through minimum inhibitory concentration (MIC) analysis. Human pathogens were more often associated with wet wipes on the beaches of the Forth Estuary than with seaweed, and there was evidence of resistance to several antibiotics. This work demonstrates that legacy plastics associated with sewage pollution can facilitate the survival and dissemination of human pathogens and as such could present an increased potential risk to human health at the beach.

A048

Investigating the function, persistence, and biosafety of novel functional constructed microbiomes for improved bioremediation of petroleum impacted soil

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Abstract

Soil is one of the most important non-renewable resources available to mankind, and the soil microbiome is made up of a diverse range of microorganisms that contribute to essential ecosystem services. The presence of recalcitrant organic compounds in fuel-contaminated soils can reduce functional redundancy within the soil microbiome, leaving a legacy of contaminated sites and posing a serious threat to the health and well-being of humans, animals, and plants. Hence, there is an immediate need to clean up (remediate) these sites. IT Carlow has developed a patented process (Ecopiling) for cleaning up contaminated soil and has developed a mixture of oil degrading bacteria (the consortium). Ecopiling is a passive bioremediation technique that involves biostimulation of indigenous hydrocarbon degraders, bio-augmentation through inoculation with known petroleum degrading consortia, and phytoremediation, which is accomplished through the effect of root growth and penetration throughout the soil and the resulting stimulation of microbial activity in the rhizosphere. This project will analyze a consortia of oil degrading bacteria for its safe use in the environment, will use -omics technology to investigate the fate of the consortia in the soil and its effects on the soil microbiome, and will determine the key genes that are involved in the oil degradation process.

A049

Pan-genome analysis of *Staphylococcus hominis* using whole genome sequences

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Abstract

Staphylococcus hominis is an opportunistic pathogen that is found on the skin of many individuals. Two subspecies Staphylococcus hominis subsp. hominis and Staphylococcus hominis subsp. novobiosepticus have been identified. Previous research by Zhang et al (2013) used multilocus sequence data to identify a polyphyletic clade suggesting that S. hominis subsp. novobiosepticus is not a true subspecies. This study used 123 publicly available S. hominis genomes, supplemented with seven commensal strains isolated from the skin of undergraduate students and sequenced with a MinION to characterize the pan-genome of the species, and investigate the phylogenetics of this species more closely. All the genomes in this study were re-annotated using Prokka, and pan-genome analysis was performed using Panaroo and significant associations in the presence of genes found using coinfinder. Subspecies of isolates were determined by assessing resistance to novobiocin and ability to ferment D-trehalose and N-acetyl-D-glucosamine All of the commensal strains were identified as Staphylococcus hominis subsp. hominis. We also created a phylogenetic tree using whole genome sequences with the aim to identify specific lineages of Staphylococcus hominis subsp. novobiosepticus.

A052

Comparative transcriptomic and physiological analysis of the extremophile microalga *Chlamydomonas acidophila* to the model organisms *Chlamydomonas reinhardtii* under copper stress elucidate key genes and physiological responses to surviving a highly metal-contaminated environment

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Abstract

Chlamydomonas acidophila is an extremophile microalga that exhibits a high tolerance to both acidity and heavy metals. PM01 is a strain of *C. acidophila* isolated from acid mine drainage systems (AMD) in the abandoned copper mine, Parys Mountain in Wales. When compared to other strains of *C. acidophila*, PM01 also exhibits a higher copper tolerance. Previous studies have shown that heavy metals can accumulate to high concentrations within the cells of PM01, however, these mechanisms of uptake and the tolerance to high quantities of heavy metals are currently unknown. Seen as important traits for the bioremediation of AMD, these qualities make PM01 an exciting candidate for further study. RNA-sequencing was conducted on PM01 and the model organism *Chlamydomonas reinhardtii*, under both a long-term and short-term copper stress investigate the transcriptomic response to excess metals. Heatmap analysis and GO-term annotation identified several processes that could underlie the physiological responses to copper, including photosynthesis and cellular transport, that could allow PM01 to adapt to a high copper environment. Further physiological studies, investigating the photosynthetic response of both species, were then conducted to complement the transcriptomic data set. Finally, several genes of interest are presented which are exciting candidates to confer a high copper tolerance. In summary, this study has provided a large data set from which important genes and processes in metal tolerance and uptake in PM01 can be elucidated. Further studies into these findings have the potential to increase the efficacy of bioremediation in AMD.

A053

No spoilers allowed: Investigating new additives to help prevent the spoilage of grass silage

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Abstract

Grass silage is grass which has been fermented anaerobically by lactic acid bacteria, acidifying it to below pH 4.5 allowing it to be stored for extended periods. Ensiling grass is an important means of preserving animal fodder throughout temperate areas. However, due to spoilage, up to 40% of grass silage produced can be lost. This is estimated to cost UK farmers £270 million pounds annually. Chemical silage additives which reduce spoilage have the potential to save farmers millions of pounds per year, reducing waste and the environmental impact of farming.

Thirteen potential additives were screened using an agar plate based assay investigating their effects on the growth and acid production of microbes isolated from Northern Irish silage in aerobic and anaerobic environments. Lysozyme induced acid production in some isolates in the both environments, while epigallocatechin gallate (EGCg), manganese sulfate and two pine tree extracts increased the proportion of acid producing isolates in the aerobic phase compared to controls, suggesting they may aid fermentation helping reduce spoilage. Interestingly two currently used additives, potassium sorbate and sodium benzoate, appear to inhibit acid producing microbes. Those additives showing increased acid production, alongside sodium benzoate, were further assessed using a vacuum pack silage model to determine their impact on silage fermentation rate, acid composition and spoilage, and on the growth of acid producing silage microbes. All additives tested had no significant effect on fermentation rate or silage stability but did significantly alter the growth and acid production of key silage microbes.

A055

Assessing the impact of microbial processes on the fate of hydrocarbons in crude oil contaminated soil from the Niger Delta

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Abstract

Oil spills are a serious problem in Nigeria's Niger delta, severely damaging the ecosystem for several decades [1]. The toxic effects of oil contamination can affect the environment including microbial diversity and processes [2] highlighted the need for remediation. However, the impact of oil contamination on the soil microbial community, and how indigenous microbes can degrade the pollutants remains largely unclear.

In this study, microbial degradation of hydrocarbons from a recent land oil spill in Ukpeliede community in the Niger delta was analysed. Geochemical analyses of soils/sediments from the contaminated area indicated the presence of hydrocarbon contamination and increased metal contamination such as chromium and nickel originating from the oil spill. Microcosm experiments showed considerable reduction in the amount of the hydrocarbon contamination present in the soil over time, particularly those with nitrate or sulfate as electron acceptors under anaerobic conditions. In addition, oil contamination clearly impacted on the microbial community structure, with dominance of *Gammaproteo-*, *Deltaproteo- Acido* bacteria, *Spirochaetia* and *Melainobacteria* noted by 16S rRNA gene sequencing in the contaminated sediments. qPCR results indicate the presence of ring hydroxylating dioxygenase genes (PAH-RHDαGN), confirming their contribution to the hydrocarbon degradation.

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A056

Quantifying the importance of plastic pollution for the dissemination of human pathogens: the challenges of choosing an appropriate 'control' material

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Abstract

Discarded plastic wastes in the environment are serious challenges for sustainable waste management and for the delivery of environmental and public health. Plastics in the environment become rapidly colonised by microbial biofilm, and importantly this so-called 'plastisphere' can also support, or even enrich human pathogens. The plastisphere provides a protective environment and could facilitate the increased survival, transport and dissemination of human pathogens and thus increase the likelihood of pathogens coming in to contact with humans, e.g., through direct exposure at beaches or bathing waters. However, much of our understanding about the relative risks associated with human pathogens colonising environmental plastic pollution has been inferred from taxonomic identification of pathogens in the plastisphere, or laboratory-based experiments on the relative behaviour of plastics colonised by human pathogens. There is, therefore, a pressing need to understand whether plastics play a greater role in promoting the survival and dispersal of human pathogens within the environment compared to other substrates (either natural materials or other pollutants). Here, we consider all published studies that have detected human pathogenic bacteria on the surfaces of environmental plastic pollution and critically discuss the challenges of selecting an appropriate control material for plastisphere experiments. Whilst it is clear there is no 'perfect' control material for all plastisphere studies, understanding the context-specific role plastics play compared to other substrates for transferring human pathogens through the environment is important for quantifying the potential risk that colonised plastic pollution may have for environmental and public health.

A058

Investigating the Presence of Plasmid Mediated Antibiotic Resistance Genes in Irish Forest Soils and River Water

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Abstract

Background: Anthropogenic activities within the environment have led to evidence that environmental habitats can act as reservoirs for antibiotic resistance genes (ARGs). These reservoirs lead to human, animal, and plant health being affected. This study focuses on plasmid mediated ARGs in Irish forest soils and freshwater rivers.

Methods: DNA was extracted from soil (n = 9) and water (n = 10) samples. qPCR was performed for 35 ARGs/mobile genetic elements (MGEs). Resulting data was analysed using Dada2 software and the relative abundance data explored.

Results: β-Lactamase, colistin, and sulfonamide resistance genes were detected in all water samples. These genes were also found in 88.89%, 55.56%, and 66.67% of soil samples, respectively. Aminoglycoside resistance was found in 60% of water and 77.78% of soil samples. A large proportion (80%) of water samples had tetracycline resistance, but none were detected within soil samples. Quinolone resistance was detected within soil and water (55.56% and 60%, respectively). We also identified vanA, drfA1, ermF, florR_1 and MGEs, IncP, intl, ORF37, and Tn5.

Conclusion: The ARGs/MGEs utilised within this study have been employed in other antimicrobial resistance studies across Europe. This study differs in that there are few publications investigating ARGs within forest soils from Europe. There are little publications from Europe assessing the resistome of freshwater rivers, as many have focused on other water sources. There is variation among the relative abundances of each sample, but there are many visible patterns of ARGs present, with multiple resistance genes appearing in many samples.

A059

Evaluating the Microbial Risks of Urban Flooding Events

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Abstract

Over the last 30 years, the frequency and occurrence of intense rainfall, and thus extreme hydrological events – such as flooding- has steadily been increasing. Most drainage infrastructure in the UK was not designed for a changing climate, and so many sewer systems in densely populated urban areas, are unable to cope– causing flooding. Sewage overflow and surface run off in urban areas can act as vectors for the dissemination of pathogens, known to cause disease among human populations. Little is understood in regards to the survivability and behaviour of a wide range of 'real life 'pathogens in different urban settings, which are fundamental to determine potential risks to public health.

Previous investigations in UK waterlogged soils have shown a clear response of microbial communities to water table variation, temperature, and nutrient availability in soil profiles. This research aims to investigate, using advanced molecular methods, the dynamics of pathogens (i.e. movement through soil, diversity, abundance, and survival rates), and microbial interactions at the soil/water interface- collecting information from field work studies and laboratory-controlled experiments.

The outcomes from this research will inform future management strategies of flooded sites that will aid to protect public health.

Thus far, despite delays due to the ongoing pandemic, preliminary work and sample analysis has been carried out, with commencement of both lab based work and field work.

A060

Microbiological Assessment of Lettuce Varieties at Farm Level and Antimicrobial Resistance Patterns of Associated Bacterial Pathogens

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Abstract

Due to the ready-to-eat state of fresh produce, consumers are predisposed to foodborne pathogens and antimicrobial resistance, a public health concern. This study evaluated the microbiological quality and antimicrobial resistance patterns of bacterial pathogens associated with lettuce varieties at farm level. Lettuce varieties (including apollo, coarse endive, iceberg, multi-red salanova and romaine cos) were examined. Fifteen samples were analysed for aerobic bacteria, yeasts and moulds, coliforms and Enterobacteriaceae while pathogen levels were determined for *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. Also, 42 isolates of the bacterial pathogens were tested for resistance to 10 antibiotics using agar diffusion method. Microbial counts for the lettuce varieties ranged from 4.0 to 7.1 log cfu/g (aerobic bacteria); 3.6 to 6.1 log cfu/g (yeasts and moulds); 4.1 to 5.8 log cfu/g (Enterobacteriaceae) and 4.0 to 5.7 log cfu/g (coliforms). Bacterial pathogen levels ranged from 2.0 to 5.2 log cfu/g, 1.7 to 5.0 cfu/g and 1.7 to 5.8 cfu/g for *B. cereus*, *L. monocytogenes* and S. *aureus* respectively. Bacterial isolates demonstrated high resistance to penicillin (100%). At least 62% of the other antibiotics were susceptible to all the bacterial pathogens except *S. aureus* with 31% susceptibility to rifampicin and *L. monocytogenes* for 43% and 56% susceptibility to piperacillin/tazobactam and trimethoprim respectively. Furthermore, multiple antibiotic resistance was revealed among the *B. cereus* (63%), *L. monocytogenes* (63%) and *S. aureus* (25%) isolates. This study has established that lettuce varieties at the farm level have high microbial levels and are potential vehicles of antimicrobial resistant bacteria.

A062

An ‘Astropharmacy’ Based on Bacterial Cell-Free Technology

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Abstract

Engineered microbes have been used as factories for manufacturing biopharmaceuticals that treat many diseases. However, such products are often temperature sensitive, typically require cold storage/transportation and have an expiry date. This approach severely limits applicability in extreme environments such as outer space and other low-resource settings, in addition to being unsustainable. One way to overcome above challenges is by removing cold storage from the supply chain and enable manufacture of biopharmaceuticals as and when needed. Cell-Free Protein Synthesis (CFPS) surpasses many limitations of cell-based expression and is evolving to be the system of choice for many therapeutic, technological and environmental applications. Owing to the vast variety of prokaryotic and eukaryotic sources of extracts, CFPS has diverse applications. We aimed to develop a more sustainable and accessible cell-free platform whereby biopharmaceuticals can be produced on-demand and on-site, by harnessing the power of bacterial cell-free extracts. We illustrate the development of an in-house cell-free system based on bacterial strain BL21 Star (DE3), further optimised for high-level chromogenic reporter expression driven by T7 polymerase. The platform was constructed by freeze-drying the cell-free components on cellulose stacks, which were layered and rehydrated with water to kickstart protein synthesis. Our results show that paper-encompassed reactions are capable of robust expression of various therapeutics following drying and rehydration, by simply changing the DNA element. Such a platform could form a part of a futuristic ***Astropharmacy*** capable of producing life-saving therapeutics in less than four hours and promote biopharmaceutical access on Earth.

A063

Soil microbiome metagenome composition in paired pasture and woodland reveals differing microbial life-history strategies

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Abstract

Soil microorganisms play a significant role in major processes driving nutrient cycling and bioavailability, therefore are essential for aboveground ecosystems. The tremendous diversity of soil microbiomes represents a great challenge to understand their complex ecology, hence it appears difficult to predict shifts in soil microbiome functions and ecosystem services they provide. To reduce complexity and improve ecological understanding, ecologist developed models like the r-K model for animals and the CSR model for plants, which proved effective in predicting general patterns of functional traits in relation to ecosystem status. Lately, the YAS model was conceptualized specifically for soil microbes, categorizing functional traits in three broad life history strategies. Here we tested its applicability in natural conditions. An extensive soil parameter characterisation was used to predict differences of YAS life strategy composition between paired pasture and woodland soils, which was tested using differential abundance analysis on soil metagenomic shot gun sequencing. The YAS model predicted that when comparing pastures to woodlands, the growth yield strategy (Y) should be enriched in pastures and the resource acquisition strategy (A) should enriched in woodlands. Our results supported a good applicability of the YAS model in natural conditions, managing to predict enriched soil microbiome functional traits according to ecosystem status. This suggested the YAS model as a promising tool to improve soil microbiome functional trait predictions in relation to environmental changes, but also highlighted theorical questions that need to be addressed to improve the model categorization of functional traits.

A065

Viunalikevirus-bacterial host interactions and potential for engineering

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Abstract

Bacteriophages are considered the most abundant biological entities on earth and powerful driving forces in the evolution of their bacterial hosts. Tailed bacteriophages have been intensively studied and they rely on tail spikes or tail fibres to recognize their cognate host receptors when adsorbing to bacteria. This adsorption step is crucial for the infection process and a key factor dictating host range specificity.

Viunalikeviruses are tailed recently classified as part of the *Ackermannviridae* family. They have dsDNA genomes of ~150Kb that exhibit extensive sequence homologies within the family, and some can effect generalized transduction. They are virulent phages and representative examples have been isolated on different Gram-negative bacteria, including animal and plant pathogens such as *Salmonella, Klebsiella, Shigella, Serratia, Kluyvera,* and *Dickeya*. There has been limited analysis of *Viunalikevirus* receptors. We analyzed the nature of the surface receptor of phage fXF4 that infects the phytopathogen, *Dickeya solani.* We investigated viral adsorption kinetics and used transposon mutagenesis, coupled with dual selection for antibiotic resistance and phage resistance, to identify mutants resistant to the virulent phage. The insertions were mapped to genomic locations, including polysaccharide production genes. The results are consistent with the view that a specific surface polysaccharide(s) is the viral receptor in the plant pathogen, as was shown previously for the defining *Viunalikevirus* (Vi-01) in the human pathogen, *Salmonella.*

We defined further the phage-host interaction between enterobacterial capsular polysaccharide (CPS) and *Viunalikevirus* adsorption *via* viral tail spikes*.* One aspiration is the development of phage-host combinations to exploit these viruses for host range extension and generic horizontal gene transfer systems.

A066

Impacts of different treatment methods for cattle manure on the spread of faecal indicator organisms from soil to lettuce in Nigeria

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Abstract

AIM - This study investigated impacts of different organic waste treatment methods on reduction and spread of faecal indicator organisms to food crops in a developing country.

METHODS AND RESULTS - Fresh cattle manure was subjected to three different treatments; anaerobic digestion, burning and composting. *E. coli,* coliforms and nitrogen content of cattle manure were measured before and after treatment in the amended soil and harvested lettuce. All treatments significantly reduced *E. coli* and coliform counts but differed in the ratio of *E. coli* or coliforms to nitrogen. Application of the recommended nitrogen dose of 120 kg ha^{-1} as bioslurry resulted in significantly lower *E. coli* and coliform contamination of soil than the same nitrogen rate applied as compost or ash. The *E. coli* content of lettuces grown on soil amended with treated wastes at recommended rates did not differ between treatments but was significantly lower than in lettuces grown on soil amended with untreated manure.

CONCLUSIONS - Treatment of manure before use as an organic fertiliser significantly reduces potential contamination of both soil and food crops with *E.coli* and coliforms. To best reduce the spread of *E. coli* from organic fertilizers, manures should be treated by anaerobic digestion.

SIGNIFICANCE AND IMPACT OF STUDY - Information from this study quantifies potential risks associated with use of manures in growing food crops by determining the ratio between pathogen content and required nitrogen application rate.

Keywords: Cattle manure, anaerobic digestion, composting, *E. coli*, coliforms, nitrogen, lettuce

A067

Microbial contamination in laser-processing environments

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Abstract

Laser technology is an important photonics tool for modifying materials and imparting them with new properties. Laser technologies are routinely applied in manufacturing various medical devices. The ambient environment in which this technology is often used could be a source of microbial contamination; however, there is limited information on the levels and types of microbial contaminants in laser-processing environments. This study aimed to determine the baseline bioburden and microbial contaminants present in three laser-processing laboratories i.e. a Clean Room (CR), a controlled Pilot Manufacturing Line (PL) and a CO_2 Laser standard laboratory (CL). Microbiological air sampling was done by passive sampling using settle plates and the microbial identity of isolates was confirmed by 16S rDNA sequencing. Each facility was monitored every week in the morning (at rest) and afternoon (during operation) for five consecutive weeks. The mean bacterial bioburden in CR at rest was 0.3±0.37 CFU/plate while CL and PL had 1.0±0.61 and 0.8±0.54 CFU/plate respectively. During operation, bioburden was 1.2±1.92, 0.4±0.2, and 0.76±0.7 CFU/plate for CR, CL and PL respectively. There was no significant difference ($p>0.05$) between bioburdens at rest and during operation within and between the three laser-processing environments. Bacterial genera isolated include *Acinetobacter, Bacillus, Dermacoccus, Micrococcus, Moraxella, Niallia, Paracoccus, Rothia, Staphylococcus,* and *Streptococcus*. The Index of Microbial Air contamination (IMA) in this study was below the maximum acceptable levels for very high-risk environments and was within the EU GMP recommended limits for microbial contamination in Grade B cleanrooms (equivalent to ISO 5).

A068

Searching for microorganisms as life signals for planetary missions

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Abstract

One of the principal objectives to planetary exploration is the search for traces of past life and evidence of conditions that may have supported life. The chances of extra-terrestrial life, if it exists on the soil of a planet, is that it will be most likely be of microbial origin and physiology. Therefore, searching for microbial signals using simple-step, ultrasensitive microbial confirmatory tests which could identify specific areas of the planet under exploration as candidates for the presence and support of life are crucial to planetary missions. In this study, we developed and optimized a miniaturized approach to assess microbial existence on Martian soil simulants.

A069

Investigation of the Metabolic Factors Influencing Anthracnose Virulence in Pepper

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Abstract

The fungal genus *Colletotrichum* is the major cause of the plant disease anthracnose, which induces the development of dark, sunken lesions on leaves, stems and fruit. *Colletotrichum* affects a wide array of cereal, fruit and vegetable crops, with many species possessing broad host ranges. For example, *C. truncatum* hosts include tomato, aubergine, soybean, lentils and pepper. Anthracnose is a particular challenge for pepper production as *Colletotrichum* thrives in the tropical conditions ideal for cultivation, with yield losses up to 80% recorded in Thailand. Currently no anthracnose-resistant pepper varieties are commercially available, so chemical control is necessary. However, resistance is emerging against existing anthracnose fungicides. Therefore, the development of new control agents is crucial to prevent future losses.

However, fungicide development is hindered by our poor understanding of the factors influencing *Colletotrichum* virulence. To tackle this issue, a panel of 22 pepper *C. truncatum* isolates collected across Southeast Asia was collated. Following assessment of virulence across three pepper species, the *C. truncatum* panel was profiled by GC-MS to identify metabolites and pathways associated with enhanced virulence. Six metabolites correlated with increased virulence were identified, including citric acid and N-acetylglucosamine. Whereas the chelation capacity of citric acid is hypothesised to improve iron uptake, the chitin monomer N-acetylglucosamine may strengthen the cell wall and has been implicated as a signal for the induction of stress and virulence genes. Furthermore, multivariate analysis demonstrated *C. truncatum* isolates were distinguishable by metabolome, suggesting metabolomic analysis could aid identification and monitoring.

A070

Development of an infection responsive coating to combat crystalline biofilm formation on urinary catheters

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Abstract

Indwelling urinary catheters are used extensively for long-term bladder management. However, they are associated with high infection rates; catheter associated urinary tract infections (CAUTIs) are the most prevalent source of healthcare-associated infection. A primary cause of catheter blockage is colonisation of the catheterised urinary tract by the urease producing bacteria *Proteus mirabilis*, which forms extensive crystalline biofilms. Blockage can initiate the onset of serious medical complications. In response, we have developed a novel infection-responsive catheter coating, to simultaneously provide early warning of blockage, and reduce crystalline biofilm formation. The coating comprises of a pH sensitive upper layer of poly(methyl methacrylate-co-methacrylic acid) and a base layer of poly(vinyl alcohol) loaded with therapeutic agents and a fluorescent dye (5(6)-Carboxyfluorescein). The elevation of urinary pH due to *P. mirabilis* urease activity results in the dissolution of the upper layer and release of the therapeutic agents and dye contained in the base layer. Release of the dye into the urine provides a visual indicator, and an early warning of potential catheter blockage. An *In vitro* model of the catheterised urinary tract was used to evaluate the efficacy of coatings loaded with either acetohydroxamic acid (a urease inhibitor) or ciprofloxacin as therapeutic agents. A significant increase in blockage time was observed when 5 mg mL^{-1} acetohydroxamic acid and 5 mg mL^{-1} ciprofloxacin were individually incorporated into the coatings. This theragnostic coating constitutes a promising strategy for the localised delivery of therapeutic agents to reduce crystalline biofilm formation and delay urinary catheter blockage.

A072

The antibacterial activity and safety of novel natural compounds as treatment options for bacterial infections

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Abstract

Objective: Fatty acid compounds and essential oils were evaluated to determine candidates with broad-spectrum antibacterial action that are safe and can be formulated as therapies against bacterial infections.

Methods: Nine fatty acid compounds and six essential oils were investigated to determine their minimum inhibitory concentrations in broth microdilution assay against Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC15442, *P. aeruginosa* Pa01*, Klebsiella pneumoniae* strain *13443*, *K. pneumoniae* strain *9633*, *Escherichia coli* strain IH3080, *E. coli* strain K1DSM, *Moraxella catarrhalis* strain ATCC 25239, Non-typeable *Haemophilus influenza (NTHi)* strain ST14, and *Neisseria gonorrhoeae* strain P9-17, and Gram-positive bacteria, *Streptococcus pyogenes* strain *8191*, Group B *Streptococcus strain* (GBS) A909, and *Staphylococcus aureus* 6571 and Methicillin resistant *Staphylococcus aureus* MRSA. The minimum bacterial concentrations were assessed, and the safety profile of promising candidates was evaluated in ocular irritation assay, and the *Galleria mellonella* assay in which bacteria infected wax worms were treated with the test compounds to prolong larval survival.

Results: The fatty acid compounds and essential oils demonstrated antibacterial action at different concentrations against all the bacteria strains. Monocaprin, octanoic acid and nonanoic acid at low concentrations (≤ 6.25 mM) and oregano oil (≤ 0.39%) had broad-spectrum antimicrobial activity against the Gram-positive and Gram-negative bacteria strains. Furthermore, oregano oil (0.39%) was safe in the bovine corneal opacity permeability (BCOP) assay and effective against *K. pneumoniae* 13443 in the *G. mellonella* assay.

Conclusion: Monocaprin, octanoic acid, nonanoic acid and oregano oil are potential candidates that can be developed into therapies to combat Gram-positive and Gram-negative bacterial infections.

A074

The ecology and antimicrobial resistance of Staphylococci colonising neonates

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Abstract

Coagulase Negative Staphylococci (CoNS) are commensals of human skin, accounting for 20% of an infant's skin microbiome. CoNS are important in promoting early innate immune responses in healthy babies but are also opportunistic pathogens responsible for 57% of Late Onset Sepsis (LOS). In neonatal intensive care units (NICUs) antiseptics are used to prevent infection of vascular catheters. Chlorhexidine (CHX) and Octenidine (OCT) are the most common agents used, but evidence is emerging of antiseptic tolerance amongst CoNS.

We undertook a longitudinal survey of CoNS from skin and rectal swabs isolated from babies in NICUs from countries with different antiseptic regimens (UK and Germany). Over 1000 isolates were characterised for antimicrobial susceptibility and sequenced. The most frequent species isolated were *S. epidermidis, S. haemolyticus* and *S. capitis,* with similar strain types present in both units. Reduced susceptibility to CHX and OCT was observed in UK isolates (where CHX is used), compared to German isolates (where OCT is used).

Investigation of the phylogeny of *S. capitis* distinguished the isolates into three clusters. The largest cluster consisted of isolates known as NRCS-A, a clone strongly associated with LOS in neonates globally. This clade contained isolates with increased MICs of multiple antibiotics and CHX, an *SCCmec* mobile genetic element and contained a CRISPR Cas type-III system.

This suggests there are different potentials for selection of resistance between antiseptics. The data also suggests genes involved in adapting to the NICU environment, the biological basis for this is continuing to be investigated

A075

Characterisation of the interaction of the predominant Avian Pathogenic Escherichia coli genotypes with cells of the avian innate immune system

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Abstract

Avian Pathogenic *Escherichia coli* (APEC) is a global health, economic and animal welfare issue which has been exacerbated by the emergence of AMR. Therefore, alternative control strategies are urgently sought. This study aimed to characterise the interaction of 11 isolates belonging to the predominant APEC genotypes with chicken 8E11 gut epithelial and HD11 macrophage-like cell lines to inform the development of broad-spectrum innate immunity-based interventions. Gentamicin protection assays demonstrated that all isolates had the ability to adhere to (measured at 2 hours post infection (hpi)), become internalised within (measured at 4 hpi) the 8E11 cells, in addition to persisting within the HD11 cells beyond 6 hpi. Significant differences (P=<0.0001) in invasion and intracellular persistence were observed between isolates, suggesting predominant APEC genotypes exhibit distinct mechanisms of interaction with HD11 cells. In addition, significant difference in nitric oxide production following infection were observed for different isolates at 4 hpi (P= <0.01) and 6 hpi (P=<0.0001). However, no significant differences were observed between isolates belonging to the same sequence type for 4/5 and 2/5 sequence types investigated, respectively. No significant differences were observed for nitric oxide production at 18 hpi with APEC (P>=0.05).

Collectively, these findings suggest that different APEC lineages have distinct interactions with the 8E11 and HD11 avian innate cell lines, pointing to different phenotypic profiles. Future studies will focus on elucidating the genetic factors influencing differential host-pathogen interactions.

A076

Evaluating the in vitro activity of the novel antimicrobial peptide IK8L against planktonic and biofilm cultures of *Staphylococcus aureus*

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Abstract

The novel antimicrobial peptide IK8L presents a promising potential additional antimicrobial agent for treatment of infection. Currently, published research into the effectiveness of IK8L against *Staphylococcus aureus* is limited.

We aimed to evaluate the activity of IK8L against *S. aureus* in both planktonic and biofilm states and compare this activity with vancomycin, a commonly used systemic agent with anti-staphylococcal activity.

A panel of 22 isolates including both reference and clinical strains were collected from Scotland and Leeds. Inhibitory and bactericidal concentrations for IK8L and vancomycin were determined using a broth microdilution assay according to EUCAST guidelines. Concentrations of 0.5X, 1X, 2X and 10X MIC were used to generate a time-kill kinetic curve for one isolate and perform a resazurin-based biofilm susceptibility assay for eight isolates.

A higher concentration of IK8L (MIC90 = 32 mg/L, range 16 – 32 mg/L) was required to inhibit the growth of our panel compared to vancomycin (MIC90 = 1 mg/L, range 0.5 – 2 mg/L). IK8L demonstrated rapid bactericidal activity similar to that of vancomycin at 2X and 10X MIC, with both agents able to reduce viable counts by >3-logs relative to the initial inoculum within 24 hours of exposure. Against biofilm cultures of *S. aureus*, IK8L exhibited comparable activity to vancomycin (49% vs 52% mean survival relative to untreated biofilms) after 24h exposure to 10X MIC.

This preliminary evidence suggests that IK8L is less potent than vancomycin, but has activity against clinical isolates of *S. aureus* in both planktonic and biofilm states.

A077

Natural antimicrobial fabrics for use within wound care management

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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 for reducing the transmission of pathogens in healthcare environments, whilst assisting the healing process and promoting localised antisepsis. This study aimed to determine the antimicrobial efficacy of natural Ugandan bark cloth which is derived exclusively from the *Ficus natalensis* and related tree species, and has been produced for generations using traditional techniques. This fabric possesses many ideal properties associated with wound dressing technology, including good gaseous transmission, biocompatibility, mechanical protection, biodegradability and cost-effectiveness. Antimicrobial susceptibility and time-kill kinetic assays demonstrated that bark cloth inhibited the growth of clinically relevant methicillin-resistant *Staphylococcus aureus* (MRSA) strains and acted as a bactericidal fabric causing a seven-log reduction in bacterial viability over a 24 h period. Scanning electron microscopy was used to reveal morphological changes in the MRSA bacterial cell ultrastructure when exposed to bark cloth, which supported a proposed mechanism of antimicrobial activity. The observed antimicrobial properties, combined with the physical characteristics elicited by bark cloth, suggest this product is ideally suited for wound and other skin care applications. This is the first example where a whole bark cloth product made by traditional methods has been employed as an antimicrobial fabric against MRSA. Bark cloth 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.

A078

The Type 3 Secretion System is a crucial mediator of macrophage-Achromobacter spp. interaction

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Abstract

Opportunistic bacteria can thrive in the immunocompromised lung of people with cystic fibrosis (PWCF), and many of these bacterial pathogens are poorly-characterised. Herein, we detail preliminary work in deciphering interactions between *Achromobacter* species and macrophages, first-line immune responders. *Achromobacter* is a Gram-negative bacterium, ubiquitous in soil but also found in hospital environments. This genus's prevalence in respiratory infection in PWCF and other immunocompromised people has sharply increased in the last decade, but little is known about the biology of infection. Many *Achromobacter spp*. are multidrug-resistant, making infections challenging to treat.

Our workhorse strains are AC055, QV306 (*A. xylosoxidans*) and AC047 (*A. insuavis*), all respiratory clinical isolates from PWCF. Macrophage response has been modelled using THP1 cells, as well as human and mouse blood monocyte-derived macrophages.

Achromobacter spp. can survive intracellularly in THP1 and primary human macrophages for several hours following phagocytosis, and bacteria accumulate in a late endosomal compartment. AC047 and other strains can induce pyroptosis (a pro-inflammatory lytic cell death) in THP1 and primary macrophages. By developing a markerless gene deletion system for *Achromobacter*, we have created mutants to demonstrate that a functional bacterial Type 3 Secretion System (T3SS) is necessary for inducing pyroptosis in infected macrophages. Moreover, wildtype but not ΔT3SS AC047 was virulent in *Galleria mellonella*. Live AC047 suppression of selected cytokine secretion in infected cells is also T3SS-mediated. We are deconvolving which macrophage inflammasome components are required for mediating this interaction, and have ruled out NLRP3. We are seeking the T3SS effector(s) responsible for triggering pyroptosis by characterising the secretome of a hyper-secreting mutant strain.

These studies demonstrate that *Achromobacter* species can survive intracellularly in macrophages, stimulate pro-inflammatory cell death, and influence cytokine release; which could inform novel treatment strategies for these pathogens.

A079

Combatting antimicrobial resistance: Small-molecule inhibitors as precision anti-virulence agents

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Abstract

Antimicrobial resistance (AMR) is an ever-increasing global problem 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 display antimicrobial activity through direct targeting of key bacterial cellular processes such as cell wall formation, which are essential for viability but are susceptible to resistance generation. In contrast, one approach to combatting AMR is the development of novel small-molecule inhibitors (SMIs) as anti-virulence agents, which target pathogen specific virulence-related traits, such as enzymes involved in the generation of post-translational modifications, biofilm formation and toxin production. This study aimed to determine the anti-virulence activity of a novel library of SMIs designed for precision targeting of essential epigenetic bacterial targets. Antimicrobial susceptibility and time-kill kinetic assays identified four lead candidates which demonstrated activity against methicillin-resistant *Staphylococcus aureus* (MRSA). SMIs were able to resolve MRSA infections in the *Galleria mellonella in vivo* model, with no observed cytotoxicity being exhibited within the larvae. Research is now focused on determining the downstream effects of these SMIs on MRSA virulence traits, such as biofilm formation and quorum sensing, in addition to confirming the bacterial cellular target site via affinity chromatography coupled with determining binding affinity. This research represents a significant advance in the search for novel antimicrobial agents which target essential bacterial processes beyond those associated with traditional antibiotics.

A080

The role of intestinal lumen signals on virulence of *Listeria monocytogenes*

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Abstract

Listeria monocytogenes is a food-borne pathogen which causes listeriosis. It is an intracellular parasite invading the epithelial cells where it escapes from the vacuole into the host cytoplasm to replicate, using actin-based motility to move within and between cells. The intracellular life cycle is well documented whereas the time spent in the lumen of the intestine is poorly understood. The aim of this study was to investigate the mechanism by which *L. monocytogenes* adapts to the environment of the small intestine prior to invasion. Specifically, to determine if the PrfA regulon, that encodes the virulence factors of *L. monocytogenes,* is switched on by signals within the intestinal lumen. Initially three signals were examined, butyrate, a short chain fatty acid molecule synthesised by bacteria within the gut microbiota, micro-aerobic (5%v/v oxygen) and serotonin (5-HT), a key neurotransmitter that modulates brain behaviour. 5-HT is secreted by enterochromaffin cells (EC) into the intestinal lumen where it acts to control gut motility, secretion and vasodilation. *L. monocytogenes* InlA strains with chromosomal *phly*::*egfp* or *pactA*::*egfp* transcriptional fusions were grown in MD10 media with two different sources of carbon either aerobically or micro-aerobically with and without 5 mM butyrate or 100 µM 5-HT and Gfp expression monitored. There was significant induction of the *phly* and *pactA* expression in micro-aerobic versus aerobic conditions. The addition of 5-HT had no effect while butyrate significantly lowered both *hly* and *actA* transcription. These data indicate that the PrfA regulon is responsive to signals likely to be encountered in the small intestine.

A081

Investigating novel putative anti-mycobacterial molecules through crystallisation techniques

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Abstract

Mycobacterial pathogens have both significant global health and economic implications. Most prominent is *Mycobacterium tuberculosis* (MTB), one of the several aetiological agents of tuberculosis (TB). MTB led in the number of deaths caused by a single infectious organism in 2019 with an estimated ten million people contracting TB, 1.7 million of these cases ending in fatality. TB also has significant zoonotic potential and is highly prominent in both agricultural and wild animal systems.

Current treatments are lengthy, and the burden of drug-resistant TB extensive. Four compounds (2-methylisocitrate- a non-cleavable ICL substrate, and CL54-01, CL-54-02 and CL-54-04 - based on a new lead chemical scaffold) have been developed to target the metabolic enzyme isocitrate lyase 1 (ICL1), an enzyme shown to be crucial for the persistence of TB infection. The inhibitory efficacy of these novel compounds against ICL1 has been assessed. Cl-45-04 is the most potent with an IC_{50} in the nM range. Initial inhibition of mycobacterial growth in complete medium has also been observed with 2-methylisocitrate and CL-54-04 inhibiting with an MIC of 1.9 mM and 100 nM respectively.

Co-crystallisation studies of protein and drug were carried out to determine the binding relationship of drug and enzyme. If the structural basis for drug efficacy can be understood, then improvements to the structure can be executed. Crystal growth was successful, and the three-dimensional structure solved, revealing movement of the active site loop in the presence of CL-54-04. Aligning with previous studies showing similar movement when inhibitors bind.

A082

Phage antibiotic synergy (PAS) – how order of administration and concentration of ciprofloxacin influences Staphylococcus aureus eradication in liquid culture in vitro?

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Abstract

We are living in post-antibiotic era where lack of efficient drug therapy increasingly affects patients worldwide. Therefore, many alternative solutions are investigated including phage antibiotic synergy (PAS). However, this approach needs deep analysis of how bacteriophages and antibiotics cooperate. In this study one of the important aspect of PAS was examined, namely how administration order of phage cocktail (vB_SauM-A, vB_SauM-D) and ciprofloxacin (various concentration) influences the efficiency of *Staphylococcus aureus* eradication in liquid culture. Overnight culture of *S. aureus* was diluted 1:100 in fresh LB medium and incubated 3 hours to gain logarithmic phase of growth. Then phages at MOI=0.1 and ciprofloxacin (concentration range 0.25mg/L – 2.0mg/L) was added simultaneously or the second component was added after 1, 2 or 3 hours. The growth kinetics was monitored for the next 21 hours, then the cells viability and phage count were analyzed. The results show that there is more than one favorable combination. The best effect was observed when ciprofloxacin 0.5 mg/L was added first, followed by phages after 1 hour (97% bacteria reduction). However, some combinations with simultaneous addition or when ciprofloxacin was added after 1 hour incubation with phages brought equally good results. Inclusion of the second healing factor after 2 or 3 hours lead to less satisfactory bacteria reduction. Interestingly, concentration of antibiotic might have impact of mechanism of synergism between phages and ciprofloxacin.

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A084

COMPARATIVE ANALYSIS OF PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LOCAL BLACK SEED (*N. sativa*) AND SPANISH OLIVE (*O. europaea*) EXTRACTS

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Abstract

This study was aimed to determine the antibacterial activity and qualitative phytochemical analysis of local cold-pressed *Nigella sativa* L. and Spanish *Olea europaea* extracts. *N. sativa* and *O. europaea* are abundantly available in various regions of India, Pakistan, and Spain respectively. Black seed extracts were obtained locally by cold-pressed extraction and commercial olive oil was purchased from Cervera, Spain. Agar well diffusion and disc diffusion methods were performed to evaluate the antibacterial effect of extracts against Gram-negative (*E. coli, P. aeruginosa*) and Gram-positive (*B. subtilis)* bacteria. Black seed oil exhibited maximum antibacterial effect as compared to olive oil. *N. sativa* extracts showed highest inhibitory action against *B. subtilis* in agar well diffusion method with zone of inhibition 12 mm as well as *P. aeruginosa* and least against *E. coli* (8 mm). Olive oil exhibited less inhibitory action comparatively as inhibition zones were only observed in disc diffusion assay where *P. aeruginosa* was reported to be most sensitive towards olive oil with an inhibition zone of 12 mm. The most effective antibacterial action was recorded against *P. aeruginosa* in both oils while *E. coli* reported as the most resistant. This is further confirmed in MIC determination. The phytochemical screening of black seed oil and olive oil revealed that the former was abundant in regard to the presence of saponins while the latter showed the absence of maximum phytochemicals responsible for inhibitory and bactericidal action.

A085

Antimicrobial activity of Ruthenium-based metallotherapeutics against *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen which is highly resistant to antibiotics and biocidal products used in both medical and industrial environments respectively.

There is an ongoing need to develop novel antimicrobial agents to combat antimicrobial resistance. Metal-based compounds have been used as antimicrobial agents throughout history for a broad range of applications. Ruthenium (Ru)-based compounds have potent antimicrobial properties and in contrast to traditional antibiotics, these compounds are thought to elicit antibacterial activity at multiple sites within the bacterial cell, which will undoubtedly reduce 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-based compounds. One lead compound was identified which was highly active at inhibiting growth of multiple strains of *P. aeruginosa* at ≤32 µg mL^{-1}. Crystal violet biofilm assays were performed which showed a decrease in biomass following exposure over a 24 h period. Scanning electron microscopy was used to reveal morphological changes in the bacterial cell ultrastructure when exposed to the Ru-based compound, with evidence of membrane perturbation which supported a proposed mechanism of antimicrobial activity. 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 such as in wound care applications.

A086

Identifying the mechanisms underpinning chlorhexidine resistance in *Proteus mirabilis*

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Abstract

Chlorhexidine (CHX) is a cationic, membrane active biocide used ubiquitously throughout healthcare. We have previously shown that the RS47 clinical isolate of *Proteus mirabilis* exhibits notably reduced CHX susceptibility (MIC >512 µg/mL). This phenotype was associated with inactivation of the *smvR* repressor and overexpression of the cognate s*mvA* efflux system. However, restoration of *smvR* activity only partially restored CHX susceptibility in RS47. To identify additional factors contributing to the CHX "tolerant" phenotype, we subjected RS47 to random transposon mutagenesis and isolated mutants with reduced CHX MICs (relative to the RS47 parental strain). One mutant (designated RS47-2) was recovered from these screens and exhibited a notably reduced CHX MIC (64 µg/mL), compared to RS47. Complete genome sequencing of RS47-2 showed a single mini-Tn5 insert in the *WaaC* gene which catalyses the addition of an L-Glycero-D-Manno-Heptose residue to the inner core of LPS, indicating that aspects of LPS biosynthesis are impaired in this mutant. Phenotypic screening of RS47-2 revealed a significant increase in cell surface hydrophobicity compared to the wildtype, indicating potential truncation of the hydrophilic O-antigen region commonly associated with a 'rough' LPS phenotype. Complementation studies revealed that restoration of smvR activity and repression of smvA in RS47-2 further reduced the CHX MIC to 16 µg/ mL. In contrast restoration of smvR activity in the RS47 parental strain only reduced the CHX MIC to 128 – 256 µg/mL. Taken together, these data show that both intact LPS and overexpression of the smvA efflux system contribute to reduced CHX susceptibility in RS47.

A087

Isolation of Antimicrobial Resistant Bacteria from Ready-to-Eat Foods in Northern Ireland

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Abstract

Antimicrobial resistance (AMR) is a global public health threat. Transmission of AMR in the food chain is poorly understood and may be an unquantified reservoir. Ready-to-Eat foods (RTEF) pose a risk as contamination will not be removed before consumption. It is routine to screen RTEF for bacterial pathogens, but isolates are not routinely subjected to AMR analysis. In this study, RTEF (n=850) were collected from all 11 local councils of Northern Ireland. Samples were tested for the presence of three organisms: *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* using selective agar and confirmatory tests under the IS0/IEC 17025:17 UKAS standard. Antibiotic susceptibilities were determined according to EUCAST guidelines, using disc diffusion methods and Minimum Inhibitory Concentrations using broth microdilution, against the following clinically utilised broad range of antibiotic agents: *S. aureus:* Gentamicin, Cefoxitin, Ciprofloxacin, Erythromycin, Fusidic Acid and Tigecycline; *L. monocytogenes:* Meropenem, Erythromycin, Trimethoprim-sulfamethoxazole and Ampicillin IV and Benzylpenicillin; and *E. coli:* Gentamicin, Imipenem, Cefoxitin, Ciprofloxacin, Ampicillin and Tigecycline. Of isolated *S. aureus* (1.8% of samples), 40% showed resistance to at least one antibiotic and 13% to three or more (multi-drug resistance). Of *E. coli isolates (2.1% of samples), 39% showed resistance to* at least one antibiotic and 11% to three or more. Work is ongoing to characterise the genotypic resistance of isolates and to complete AMR analysis for isolated *L. monocytogenes*. This study demonstrates a potential pathway of the transmission of multidrug resistant bacteria in the food chain and suggests that AMR screening should be routinely completed on RTEF.

A088

Evaluation of the cross-protective efficacy of avian pathogenic *Escherichia coli* mutants with truncated lipopolysaccharide in chickens

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Abstract

Respiratory and systemic infection caused by avian pathogenic *Escherichia coli* (APEC) represents a major problem in the poultry industry due to mortality, carcass condemnation and reduced meat and egg production. The most common serogroups associated with avian colibacillosis worldwide are O1, O2 and O78. Control by vaccination can be challenging due to the high variability and antigenic dominance of the lipopolysaccharide (LPS) O-antigen which impedes cross-serogroup protection. We hypothesised that truncation of the lipopolysaccharide may expose underlying surface antigens that could enhance cross-protection. We constructed two mutants of an APEC O78 strain. These were designed to disrupt the synthesis of the O-antigen monomers (*Δrfe*) or their polymerisation (*Δrfc*), while keeping the core of the LPS intact. White Leghorn birds divided into groups of 5 animals, were orally vaccinated with APEC *Δrfe*, APEC *Δrfc* or APEC*::pgl* (previously reported to protect against homologous infection) and challenged via the intra-airsac route with either APEC O78, O1 or O2. While the protection conferred by APEC*::pgl* to homologous O78 challenge was reproduced, it did not protect against the heterologous strains. Furthermore, none of the vaccine candidates with truncated LPS conferred protection to any of the challenge strains. These data suggest that despite our efforts to identify new conserved vaccine targets, protection against experimental APEC infection seems to be mostly mediated by host responses to the O-antigen.

A089

Drug repurposing approaches to target bacterial cytochrome bd oxidases

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Abstract

Cytochrome *bd* complexes are terminal respiratory oxidases found exclusively in the aerobic respiratory chains of prokaryotes that generate a proton motive force by coupling quinol oxidation to the reduction of dioxygen. Previous work has demonstrated that cytochrome *bd* complexes are important during infection for a variety of bacterial pathogens, including *E. coli* and *M. tuberculosis*, demonstrating their potential as drug targets. Herein, *in silico* tools were used to screen a library of approved drugs for their ability to inhibit cytochrome *bd*-I from *E. coli*. In order to investigate the efficacy and specificity of the top hits, mutant strains of *E. coli* that express either cytochrome *bd*-I or cytochrome *bo*' as the sole respiratory oxidase were used as a test system, and the expected spectral signals of these respiratory oxidases were confirmed for these strains using difference spectroscopy. Membranes were isolated from these strains, and candidate drugs from the *in silico* analyses were tested for their ability to inhibit oxygen consumption by cytochrome *bd*-I or cytochrome *bo*' using an oxygen electrode. Selected drugs were identified as inhibitors of cytochrome *bd*-I, and further work has been undertaken to aid our understanding of their mechanisms of action and potential for broader applications in antimicrobial chemotherapy.

A090

Graphene Family Materials as Novel Chronic Wound Therapeutics

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Abstract

The most common organism in chronic wound infection, *Pseudomonas aeruginosa*, is multidrug resistant and biofilm forming, rendering antibiotics ineffective. Novel antimicrobials are therefore urgently needed. Graphene oxide (GO) has been reported as antimicrobial however, variability of production, processing and testing methods in the literature that influence pH, particle size and dispersity of GO, instil a lack of confidence in this hypothesis.

We aimed to develop a robust method for assessing antimicrobial activity of GO in standard microdilutions against *P. aeruginosa* (PA01). Raman spectroscopy, zeta potential, particle size and polydispersity were used to assess GO throughout.

Industrially manufactured GO pH was 1.80 and water washing achieved pH 4.16. Neutralisation with NaOH required subsequent water washing to prevent salt-induced aggregation and remove manufacturing contaminants. We established that NaOH neutralisation followed by three washes to remove salts was sufficient to generate GO of good quality. Our clean GO was lyophilised to concentrate, then resuspended in water and dispersed by sonication for use in MIC assays. GO aggregation due to media constituents was observed in MIC assays. We established PA01 viability in deionised water over 24h and used water in subsequent assays. Initial investigations showed that treatment with GO up to 5 mg/mL had a maximum 2.25 log reduction in CFU/mL after 6h, with an apparent dose-dependent effect.

Antimicrobial activity assays are ongoing. We hypothesise that the cleaned, neutralised GO will allow material assessment in the absence of interfering substances, yielding a robust assessment of GO's antimicrobial activity.

A091

Probing the *Clostridioides difficile* – host interface using innovative gut mimics

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Abstract

Clostridioides difficile is a significant cause of hospital-acquired gastrointestinal infections in humans. The recent rise of community-acquired *C. difficile* has increased the burden on global healthcare services due to inherent antibiotic resistance paired with its propensity for recurrent infections.

C. difficile colonisation of the gut is an essential determinant of bacterial carriage and disease outcome; however, we lack knowledge about bacterial or host factors modulating interactions between *C. difficile* and the gut epithelium. The gaps in our understanding partly attribute to the lack of tools that accurately represent the gut environment whilst allowing real-time monitoring of infection progression.

We have recently performed a dual RNA-seq analysis of *C. difficile* infection in an in vitro human gut model, which revealed changes in expression of several bacterial and host genes. Here we will present data investigating the functions of cell wall associated genes altered during infection using isogenic mutants and in vitro and in vivo infection models. Host genes that are modulated during infection, such as the mucin genes, are also being investigated further with KO cell lines, to explicate their role in *C. difficile* infection (CDI). Concurrently, we have redesigned the current static in vitro gut model to introduce flow to better mimic gut physiology. The result will be a beneficial tool to elucidate the underpinning mechanisms of CDI and inform new therapeutic and diagnostic development.

A092

Anti-virulence and anti-biofilm factors elaborated by Escherichia coli Nissle 1917

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Abstract

In the quest for mitigators of bacteria virulence, we have investigated the effects of supernatants of *E. coli* Nissle against *P. aeruginosa*. Cell-free supernatants (CFS) of *E. coli* Nissle had a significant ($P<0.05$) protective effect in a *Galleria mellonella*-based larval virulence assay, with 80% protection of the larvae when administrated 24 h prior to challenge with the *P. aeruginosa*. *E. coli* Nissle CFS was also tested for antimicrobial and antibiofilm activity against *P. aeruginosa*. *E. coli* Nissle CFS significantly ($P<0.05$) inhibited bacterial adhesion to abiotic surfaces and dispersed mature pseudomonas biofilm without inhibiting bacterial growth. Characterization of the putative antibiofilm and anti-virulence compound indicates the involvement of proteinaceous factors . In summary, *E. coli* Nissle 1917 CFS is a potential source of proteinaceous antibiofilm and antivirulence compounds.

A093

How do droplets containing bacteria and viruses dry on non-porous materials, and why is it important?

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Abstract

Antimicrobial materials are becoming more popular as a method to control microbial survival and growth. Conditions for standardised test methods used for efficacy assessment are not aligned to end-use scenarios, particularly with regards to the presence of moisture. As moisture can be critical to antimicrobial activity, it is important to understand the relationship between moisture, microorganism, and surface to enable improved test method specification. Thus, the impact of different environmental conditions (humidity, temperature) on evaporation time of droplets (1ul and 5ul) containing bacteria or bacteriophage was assessed. Environmental factors significantly affected the evaporation of the droplets in all cases through physical factors such as changing the diffusion gradient of water across the droplets free surface (interface between liquid and surrounding gas). The addition of bacteria and bacteriophage increased the evaporation rate via particle/cell deposition at the outer edge of the droplet following a change in the evaporation mode, a phenomenon known as the 'coffee ring' effect. The addition of bacteria provided more statistically significantly instances of increased evaporation rate than bacteriophage. SEM imaging revealed the size and density of bacterial deposition that allowed the quantification of the coffee ring effect. All of these factors have a significant effect on the evaporation time of a droplet and potentially the antimicrobial efficacy of a material.

A094

Development of a rapid method for assessing the efficacy of antibacterial photocatalytic surface coatings

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Abstract

Antimicrobial resistance is one of the most important emerging challenges for global health. Furthermore, the ability for microbial species to colonise surfaces, subsequently form complex biofilms, and undergo horizontal gene transfer represents particularly pressing issues in the spread of both clinical and environmental antimicrobial resistance.

Approaches such as visible-light activated photocatalytic coatings may represent an attractive antimicrobial and antibiofilm solution. However, determining the antibacterial effects of photocatalytic coatings is time consuming and requires specialist expertise, representing a barrier to entry; a method that enables rapid screening of coatings for photocatalytic-antibacterial activity would be beneficial. As viable microorganisms to reduce the dye resazurin from a blue to a pink colour, and using an inexpensive digital camera to identify the time of the colour change, the method relates the time taken to detect this colour change with number of viable microorganisms.

The antibacterial activity of two photocatalytic materials (Bi_2O_3 and TiO_2) were screened against two pathogenic organisms (*Escherichia coli* and *Klebsiella pneumoniae*) using traditional testing and enumeration techniques (BS ISO 27447:2009) and the novel rapid method.

Bismuth oxide had excellent antibacterial activity after 60 minutes under ambient visible light against *E. coli*, but was less effective against *K. pneumoniae*. Encouragingly, the rapid method showed excellent agreement with existing tests in terms of number of viable cells recovered. Due to advantages such as low cost, high throughput, and less reliance on microbiological expertise, this method is recommended for researchers seeking an inexpensive first-stage screen for putative photocatalytic-antibacterial coatings, especially in resource-lean environments.

A095

Intra-colony channel morphology in *Escherichia coli* biofilms is modulated by radial position, nutrient availability and substrate stiffness

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Abstract

Nutrient-transporting channels have been previously identified in biofilms of *Escherichia coli*, however the effect of environmental conditions on intra-colony channel architecture is poorly understood.

We used fluorescence mesoscopy, which provides sub-micron resolution within millimetre-size live biofilms, to determine the influence of nutrient availability and substrate stiffness on intra-colony channel morphology. It was found that channel width increased non-linearly with radial distance from the centre of the biofilm irrespective of the nutrient availability, and that channels were proportionally narrower at the centre of the biofilm with respect to the edges. We hypothesise that this happens to create a more ramified network of channels at the centre of the biofilm, which facilitates the transport of nutrients to constituent cells. Absolute channel width was also affected by the nutrient concentration in the substrate: channels forming on carbon-limited substrates were on average 50% wider than those forming on nitrogen-limited substrates. Finally, substrate stiffness was found to affect intra-colony channel cell packing inside biofilms grown on rich medium substrate. It was also observed that channels in colonies grown on minimal media were larger and had edges that were more clearly resolved.

These findings suggest that intra-colony channel architecture in *E. coli* biofilms is intrinsically linked to substrate composition and nutrient availability.

A096

A *Candida parapsilosis* secreted factor inhibits *Staphylococcus aureus* biofilm formation

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Abstract

Hospital-acquired infections are often biofilm in nature. Biofilms commonly associate with medical devices which result in the need for the replacement or removal of the device. These infections are extremely difficult to treat as biofilms are often polymicrobial, containing both bacterial and fungal species. Relatively little is known about the complex mechanisms that regulate these interspecies interactions. Previous studies have focused on the synergism observed between S*taphylococcus aureus* and *Candida albicans* during polymicrobial biofilm formation. In this study we investigated the effect of *Candida parapsilosis* on *S. aureus* biofilm formation. We have demonstrated that growing *S. aureus* in the presence of *C. parapsilosis* cells or cell-free supernatant reduced bacterial biofilm formation. This suggests that *C. parapsilosis* is secreting a factor that can inhibit *S. aureus* biofilm formation. This factor does not kill *S. aureus*. The *C. parapsilosis* supernatant has no effect on *S. aureus* biofilm attachment but significantly reduces *S. aureus* biofilm across different time points, including the reduction of preformed biofilm. Our data indicates an antagonistic relationship between the two pathogenic species.

A097

Investigating biofilm matrix composition in *Bacillus subtilis*

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Abstract

Microbes have the capacity to form structures known as biofilms; communities of cells surrounded by a self-produced matrix that mainly incorporates extracellular DNA, exopolysaccharide (EPS) and proteins. Biofilms are ubiquitous, found in natural and artificial environments, and serve to protect the population harboured within. Biofilms are highly diverse, possessing characteristics that have wide ranging implications for industrial and clinical settings. Understanding biofilm composition and response to environmental conditions strengthens our ability to harness or control biofilms. The bacterial species, *Bacillus subtilis*, is widely used for biofilm investigation and has industrial applications. The established components contributing to structure are EPS, the proteins TasA and BslA, and to some extent poly glutamic acid (PGA). We have shown that *B. subtilis* strain NCIB 3610 can alter its matrix composition in response to environmental conditions and with increasing temperature switches to a matrix dominated by PGA. However, the mechanism by which this switch in matrix types is achieved is unknown, as is the fate of the other matrix components that give rise to the structure and normal function of the biofilm and whether this mechanism can be found in other strains or species. In this investigation we are exploring these questions and narrowing down the search for the temperature-controlled mechanism for altering the biofilm matrix.

A098

Development of a tubing colonisation model to study biofilm formation on silicone tubing by Acinetobacter baumannii

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Abstract

Introduction

A. baumannii is typically underrepresented as a uropathogen, but it does have the ability to cause catheter associated urinary tract infections by forming biofilms on catheter tubing. Investigations of biofilm formation are often carried out in microtiter well plates, which do not accurately represent the environment. To better explore the formation of *A. baumannii* biofilms in catheter tubing, a model was developed to explore the colonisation of silicone tubing in clinically relevant media.

Methods

To analyse biofilm formation of an MDR strain of *A. baumannii*, a modified Drip Flow Biofilm Reactor® was used as a basis for a tubing colonisation model, with the flow of modified Artificial Urine Media at 720ml per day as the clinically relevant nutrient source. The system was run for five days to allow for full development of a mature biofilm.

Results

Analysis of the biofilms involved quantification via live/dead staining and viable counts, and also imaging via SEM. Biofilms grown in mAUM appeared to have less biomass under SEM than those grown in MHB, with biofilms grown in the rich media forming structures typical of mature biofilms. The viable counts and live/dead staining did not reveal any significant differences between biofilms grown in different media types.

Conclusion

This tubing colonisation model provides simple methods to analyse biofilm formation and also the potential of adaptation to better mimic the bladder environment with the presence of a catheter.

A099

Disruption of efflux activity reduces biofilm formation through multiple pathways

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Abstract

Bacteria mostly exist in aggregated communities called biofilms. Biofilms are associated with persistent infections, tolerance to antimicrobials and poor patient outcomes. Therefore, there is much interest in anti-biofilm therapeutics. We previously identified that inactivation of efflux pump activity reduced biofilm formation through transcriptional repression of *csgD*, a regulator of curli biosynthesis, a major component of the biofilm matrix for many pathogens, such as *E. coli*, *Salmonella enterica* and *Pseudomonas aeruginosa*. However, the exact pathway through which efflux activity affects biofilm formation is unknown. Our aim was to address this by identifying all genes involved in both biofilm formation and efflux activity in *E. coli* and *Salmonella* Typhimurium. We used TraDIS-*Xpress*; a massively parallel transposon mutagenesis approach using transposon-located promoters to assay both essentiality and impacts of altered expression of all genes in the genome. We found genes involved in both efflux activity and biofilm formation had roles in respiration, DNA housekeeping, translation, signalling, purine biosynthesis, transcriptional regulation and protein chaperoning. We identified two pathways for further investigation, including the *nuo* operon encoding NADH dehydrogenase at the beginning of the electron transport chain and *maoP* involved in Ori domain organisation. We hypothesise that deletion of the *nuo* operon reduces curli biosynthesis through disrupting the proton gradient, and that deletion of *maoP* reduces curli biosynthesis through its interaction with c-di-GMP. These hypotheses were investigated to further characterise the link between efflux activity and biofilm formation to deepen our understanding of mechanisms by which both efflux expression and biofilm formation are regulated.

A100

Comparison of the genetic basis of biofilm formation between *S*. Typhimurium and *E. coli*

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Abstract

Biofilms complete a lifecycle where cells aggregate, grow and produce a structured community before dispersing. Progression through this lifecycle requires controlled temporal gene expression to maximise fitness at each stage. We recently described the genes required for biofilm formation in *E. coli* over time and showed how temporal control of gene expression is necessary for optimal fitness across the biofilm life cycle. This work used TraDIS-*Xpress*; a massively parallel transposon mutagenesis approach using transposon-located promoters to assay both essentiality and impacts of altered expression of all genes in a genome. We have repeated this work with *Salmonella enterica* serovar Typhimurium allowing a comparison between species. This revealed a core group of genes and pathways required for biofilm development in both species, such as those involved in matrix production, flagella biosynthesis, LPS production and similar transcriptional regulators. We also found several species-specific pathways: genes with roles in in c-di-GMP metabolism and DNA housekeeping affected only *E. coli* biofilm formation, and genes with roles in cAMP biosynthesis and amino acid biosynthesis affected only *S*. Typhimurium biofilm development. Our most prominent finding was that genes involved in respiration made a considerable contribution to the fitness of *S*. Typhimurium in the mature biofilm, including 10 out of 14 genes in the *nuo* operon. Finally, we identified 21 genes with no previous connection to biofilm formation affected the fitness of *S*. Typhimurium growing in a biofilm. This work builds upon our knowledge of the core requirements for successful biofilm formation in Enterobacteriaceae through time.

A101

The biofilm lifestyle defines evolutionary trajectories to antibiotic resistance in *Pseudomonas aeruginosa*

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Abstract

The selective pressures intrinsic to the biofilm lifestyle and the role of their physiological distinctions in modulating evolutionary outcome remains poorly understood. This work uses experimental evolution to study lifestyle-contingent evolutionary trajectories to antibiotic resistance in *Pseudomonas aeruginosa* adapted to ciprofloxacin, ceftazidime and tobramycin. Over the course of 18 transfers, biofilm and planktonic lineages were serially passaged under antibiotic stress which doubled every three transfers from 0.25× to 8× MIC. Every 6 transfers, the lineages were genome sequenced and phenotyped for changes in relative fitness, biofilm formation and antibiotic susceptibility.

In both lifestyles, adaptation to antibiotics selected for the same 'driver' mutation: *gyrA, ampR* and *fusA1* in ciprofloxacin-, ceftazidime- and tobramycin-adapted lineages, respectively. Moreover, in the absence of stress, serial passage of biofilms selected for biofilm hyperproduction mediated by mutations in the phosphodiesterase *dipA* and the *yfiBNR* c-di-GMP signalling complex. However, biofilm hyperproduction was not selected in antibiotic-adapted lineages but, by virtue of the repertoire of secondary mutations selected, lineages demonstrated lifestyle-specific evolutionary trajectories to resistance. Ciprofloxacin selected for loss-of-function of type IV pili biogenesis and alginate biosynthesis exclusively in planktonic lineages. Moreover, ceftazidime-adapted biofilm lineages possessed mutations in the peptidoglycan biosynthesis genes *mpl* and *dacB* not observed in planktonic lineages. Finally, tobramycin-adapted planktonic lineages possessed mutations in regulators of MexXY and the osmoporin *mscL* which were absent from biofilms. Therefore, this work provides novel insights into the central role of lifestyle in the evolution of resistance and identifies selective targets under differential selection between lifestyles during adaptation to antibiotics.

A102

The effect of glucose on *Streptococcus mutans* invasion of an *in vitro* synthetic community of oral bacteria

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Abstract

One of the roles of a healthy oral microbiome is to impede colonisation by pathogenic species [1]. This work aimed to understand and quantify the functions underpinning colonisation of the oral microbiome by the cariogenic pathogen *Streptococcus mutans*.

The invasion of *S. mutans* into a pre-formed, synthetic 4-species oral bacterial community in low and high glucose environments was investigated. The community was grown in a chemically defined medium in a CDC bioreactor, which contained hydroxyapatite coupons for biofilm growth to mimic tooth enamel. The medium was fed continuously at 0.4 mL min^{-1}, representative of salivary flow. Biofilms were imaged using fluorescent *in situ* hybridization (FISH), species were quantified using qPCR.

S. mutans easily invaded the oral synthetic community under the high glucose condition, seven days after inoculation, *S. mutans* constituted over 99% of the biofilm population. In the low glucose condition, only 3% of the biofilm consisted of *S. mutans* at the same timepoint. The pH of the bulk medium stabilised at 5.3 at the high glucose concentration, the value increased to 6 when glucose was limited. Direct visualisation by FISH showed that biofilms shifted from a more balanced cohort of bacteria under low glucose conditions, to a community dominated by streptococci at high glucose concentrations.

To conclude, we developed an *in vitro* model of the oral cavity and demonstrated that a high glucose concentration enabled *S. mutans* to outcompete other oral bacteria in the biofilms leading to a low pH environment, as seen *in vivo* [2].

[1] Doi:10.1128/CMR.00051-19

[2] Doi:10.1111/j.1834-7819.2008.00064.x

A103

Phenotypic and genotypic characterisation of colony morphology variants generated in a biofilm model in the presence or absence of the preservative sodium benzoate

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Abstract

Pseudomonas aeruginosa, the most common contaminant within HPC products and it is problematic due to its nature as an antibiotic resistant opportunistic pathogen. This has caused concerns among manufacturers about the robustness of new strategies compared to those used traditionally and how this impacts the adaptation of contaminants such as *P. aeruginosa.*

Using a directed evolution method, we showed that *P. aeruginosa* adapts when exposed to moderate levels of sodium benzoate (NaB). Biofilms were established on stainless steel beads and successively transferred in two static levels of NaB. Twelve endpoint isolates were selected to characterise both genotypically and phenotypically to further understand the role that NaB exposure plays in *P. aeruginosa* adaptation in biofilms. Populations in both the control group and NaB groups diversified by colony morphotype as the experiment progressed. Most isolates selected (11/12) showed an increase of 2x the ancestral Minimum Inhibitory Concentration, however, this was irrespective of treatment group. Isolates were also tested for changes in attachment phenotypes using gold-standard crystal violet assays. Whole-genome sequencing revealed mutations associated with adaptation to biofilm lifestyle and parallel evolution in several genes, namely, *wspA, wspF,* and *dipA* as well as parallelism in putative function associated with cyclic-di-GMP regulation.

This study is the first to show the types of evolutionary strategies that *P. aeruginosa* adopts in the presence of NaB preservative stress. This study highlights the importance of biofilms under these conditions and that the majority of adaptations that *P. aeruginosa* underwent were related to its lifestyle within biofilm populations.

A104

Electrochemical testing of anti-biofilm metabolites extracted from Scottish marine sediment microorganisms

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Abstract

Antimicrobial resistance (AMR) causes more than 700,000 deaths per year and is projected to increase to more than 10 million by 2050. Bacterial biofilm formation is a major concern, as bacteria within biofilms have shown resistance to 100-fold higher concentrations of antibiotics than planktonic cells. This is due to the strain entering a dormant-like state, reducing its growth rate. As many antibiotics target mechanisms of active metabolism, they are less effective. Therefore, new antibiofilm-metabolites are needed to inhibit formation and target established biofilms. Bacteria from the marine environment are a rich, untapped source of novel bioactive metabolites, many of which have not been tested for antibiofilm properties. In this work, we have isolated 12 actinomycetes from Scottish marine sediments and extracted their metabolites from liquid culture. These metabolite extracts have been quantified for their ability to prevent *Pseudomonas aeruginosa* (PA14 & LESB58) biofilm formation using square wave voltammetry (SWV), and the method validated using crystal violet staining. An increased measurement in SWV current amplitude (µA) indicates biofilm formation. Control samples measured with SWV (0.02 V, 4 hours) showed an increase in current (5.9-45.9 µA), while those exposed to 1 mg/mL of actinomycete metabolite extract showed no significant change. Our results show that we have successfully extracted an antibiofilm agent which is active against *P. aeruginosa* biofilm formation, and this can be quantified using SWV. Next, *P. aeruginosa* biofilms will be formed on medically relevant materials, and the ability of the metabolite extracts to reduce these biofilms will be measured using SWV.

A105

Disruption of the *dnaK* gene in *Clostridioides difficile* leads to significant changes in the transcriptome of planktonic cells and increased biofilm production

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Abstract

Clostridioides difficile, named an 'Urgent threat' by the CDC in 2019, causes the most common healthcare-associated bacterial infection in the USA. The *dnaK* gene, found to be involved in the heat stress response encodes a molecular chaperone, DnaK, that is responsible for protein folding. ClosTron disruption of the *dnaK* gene *C. difficile* 630Δ*erm* resulted in a mutant strain (630Δ*erm:dnaK*) with 50% cell elongation, increased cell hydrophobicity, and increased biofilm production.

Biofilm formation in 630Δ*erm:dnaK* was investigated using microtiter plates, glass coverslips and semi-permeable membranes and was quantified by crystal violet, 2,3,5-Triphenyltetrazolium chloride (TTC) assays, viable cells counts and BacLight staining as appropriate. 630Δ*erm:dnaK* biofilms grown in microtiter plates and glass coverslips were significantly larger than those formed by the parent (630Δ*erm*) and wildtype (630) controls.

RNAseq analysis of the 630Δ*erm:dnaK* mutant revealed significant differences in gene expression in planktonically grown mid log phase cells (6 h, 37°C) compared to 630Δ*erm*. Other genes involved in the heat stress response (*groL* and *groS*) showed a 6-fold increase in expression ($p < 0.001$) whereas motility and chemotaxis related genes, which are known to heavily influence biofilm formation, exhibited an overall decrease in expression in the *dnaK* mutant. These observations corroborate the non-motile phenotype exhibited by 630Δ*erm:dnaK* and the lack of flagella revealed by TEM.

Taken together, our data suggest that the phenotypic differences and increased biofilm formation between 630Δ*erm:dnaK* compared to 630Δ*erm* and 630, are underpinned by the significantly altered transcriptomic profile in the mutant, and suggests a pleiotropic role for the *dnaK* gene in *C. difficile* 630.

A106

Characterising A Novel Antimicrobial Sol-Gel Device Coating

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Abstract

Periprosthetic joint infections (PJI) affect 1-3% of primary joint replacement surgeries and have a devastating impact on patient's wellbeing. They pose a treatment challenge due to growth of antimicrobial resistant biofilm on implant surface material and surrounding tissues, with treatment typically involving invasive revision surgeries and systemic antibiotic treatment. Alteration of implant surfaces to prevent biofilm formation is a major research focus at present, including the use of antimicrobial coatings.

This study characterises a range of clinically relevant antimicrobials including gentamicin, clindamycin, vancomycin and caspofungin from a novel, ultrathin sol-gel coating for prosthetic devices. The sol-gel coating showed very low cytotoxicity levels against human cells and the release of antibiotics demonstrated by elution assays and detected using LC-MS. Antimicrobial sol-gel activity was shown against a panel of clinically relevant bacterial and fungal strains using disc diffusion assays. Broth microdilution and culture methods were used to determine MIC and MBC values and the Calgary Biofilm device method was used to determine anti-biofilm activity of the coatings.

This research forms part of an extended study into a very promising new antimicrobial delivery strategy to prevent PJI, thus reducing the future burden upon healthcare costs and patient wellbeing.

A107

Methyltransferase BrxX (PglX) defines the site-specificity of the Type I BREX defence system from *Escherichia coli* HS

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Abstract

Bacteriophages are intracellular bacterial parasites that proliferate by exploiting resources of their hosts. Bacteria have evolved multiple ways to defend themselves from phage predation. We investigate the mechanism of host protection afforded by one such defence system - BREX. The Type I BREX is comprised of six proteins with unconfirmed functions. Similar to some restriction-modification (R-M) systems, BREX methylates specific asymmetric sites in the host genome to distinguish self from the invading viral DNA. In contrast to the R-M systems, the mechanisms of the phage infection "exclusion" mediated by BREX are unknown.

Whatever the mechanism of BREX defence, it should be initiated with the recognition of non-methylated BREX sites in the phage genome at the early stages of infection. Therefore, we investigated the interaction of BREX proteins with DNA. *In vitro*, BrxX (PglX) protein, an adenine methyltransferase, binds dsDNA in a sequence-specific and SAM-dependent manner. Using a variant of a ChIP-Seq assay we demonstrate *in vivo* BrxX binding to the BREX sites in the phage T7 genome at the early stages of infection. Thus, the binding of BrxX methyltransferase must define the site-specificity of the BREX system. We also investigate the mechanism of BREX defence inhibition by a DNA-mimic protein Ocr encoded by phage T7. Ocr completely disrupts BrxX binding to DNA *in vitro*. Thus, the anti-BREX function of Ocr is accomplished by preventing BrxX-mediated recognition of DNA with unmethylated BREX sites.

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A108

Investigating CRISPR/Cas adaptation mechanisms

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Abstract

CRISPR/Cas is a prokaryotic adaptive immune system that utilizes a protein complex, Cas1-Cas2, to capture and integrate DNA from mobile genetic elements (MGEs). This generates specialised chromosomal loci (CRISPRs) that are the basis of immunity. There is detailed knowledge of Cas1-Cas2 structure and function except that we do not know how the DNA is identified and thus captured for establishing immunity against an unfamiliar MGE, a process called naïve adaption.

We are investigating this question, how DNA captured, using *E. coli* as a model organism. One hypothesis we are pursuing is the involvement of *E. coli* chaperone proteins – these proteins are co-opted by some MGEs for initiating MGE replication. We present our latest genetic and biochemical data that implicate chaperones as part of naïve spacer acquisition, by guiding Cas1-Cas2 to MGEs that are initiating replication.

A110

Type I-U CRISPR system: An uncharacterised CRISPR with novel components

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Abstract

To date, CRISPR systems have been widely discovered and applied in different microbiological fields. However, there is always an uncharted area of CRISPR systems in nature, awaiting exploration. The type I-U CRISPR system is one of the subtypes of type I CRISPR systems (the best understood being the type I-E system from *E. coli*). Characterised by the enigmatic *cas* proteins Csb2 and Csx17, the type I-U system possesses a unique mechanism in CRISPR recognition and interference. Here, we expressed and reconstructed a type I-U system from *Thioalkalivibrio sulfidiphilus*. We present key insights into the biochemistry and mechanism of the system, and a first view of the structure on the effector complex of type I-U is provided. Heterologous expression in *E. coli* provides immunity against mobile genetic elements. These observations provide an overview of the type I-U system, potentiating fundamental studies and further applications.

A111

Furaneol and Sotolon are naturally occurring inhibitors of biofilm formation in the chronic wound pathogen *Pseudomonas aeruginosa*

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Abstract

Eradicating *Pseudomonas aeruginosa* biofilms in chronic wounds is a significant healthcare challenge. One method of achieving this is *via* disruption of quorum sensing (QS), the process that governs biofilm formation. This work used structural homologues of the QS signalling molecules to inhibit communication.

Furanones are naturally occurring chemicals with a high degree of structural similarity to N-acyl homoserine lactone (AHL) signalling molecules used by *P. aeruginosa*. This allows furanones to competitively bind to the AHL receptor, thus blocking detection.

Here, we demonstrated that furaneol and sotolon, were able to significantly limit biofilm formation when sub-inhibitory concentrations were applied directly during at the point of inoculation, reducing biofilm biomass by up to 88.3% and 87.2% respectively when compared to untreated controls. When applied to 24 and 48 h old biofilms furaneol and sotolon reduced total biofilm biomass by up to 67.1% and 77.7%, respectively.

To show the potential of furanones for use in wound dressings, furanone-loaded aerogels were assessed using a novel *in vitro* model of chronic wound biofilm. Sotolon-loaded aerogels achieved a 3.65 log reduction in viable, biofilm bound cells after 24 h. We compared this to clinically relevant antimicrobial dressings. Sotolon loaded aerogels were superior to Inadine (2.54 log decrease), Telfa AMD (3.88 log increase), and Actilite (no effect). The furanone aerogel was as effective as, Aquacel AG.

These data strongly support the potential for furanones in combatting wound biofilm. Furthermore, our novel aerogels show promise as wound dressing materials, and for the application of antimicrobials to infected wounds.

A112

Bacterial Chat: Detecting quorum-sensing-related molecules in Oral bacteria, using bacterial biosensors

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Abstract

Oral bacteria exist within multispecies biofilms, commonly referred to as dental-plaque. The formation of mature plaque involves quorum sensing (QS) molecules. QS is the cell-to-cell communication between microorganisms mediated byrelease and detection of small signalling molecules. Such molecules coordinate various activities within oral biofilms. The persistent presence of plaque can initiate periodontal diseases (gum disease) and QS molecules may play a vital role. There is a paucity of data on QS in periodontal disease pathogenesis, therefore the aim of the present work is to identify the major quorum-sensing molecules produced by oral bacteria and explore their pathogenic role.

Amongst the many bacterial signalling molecules described today, AutoInducer-1 and -2 are of particular interest here. The cross-species signalling molecule AutoInducer-2 (AI-2) is detectable by reporter strain Vibrio harveyi, which expresses a luminescence gene upon AI-2 exposure. This bioluminescence can be quantified and correlated to AI-2 production by different oral bacteria, but also under different conditions, such as co-culturing of different species, which makes it highly relevant to the polymicrobial oral biofilms.

AutoInducer-1 or acyl homoserine lactones (AHLs) are QS molecules produced by Gram-negative bacteria. Currently, AHLs are considered to play a minor role in plaque development, yet their production by oral bacteria has not been fully explored. Various methods exist for detecting AHL molecules, including Thin Layer Chromatography (TLC) or bioluminescence.

Using a selection of oral plaque bacteria, from early to late colonisers, assays to detect AI-1 and AI-2 production are being carried out and initial findings will be reported.

A113

Atomic-scale interactions between quorum sensing autoinducer molecules and the mucoid *P. aeruginosa* exopolysaccharide matrix

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Abstract

The lungs of cystic fibrosis (CF) patients are prone to chronic infection by mucoid *Pseudomonas aeruginosa* biofilms, whose chronicity is associated with the formation of cation cross-linked exopolysaccharide (EPS) matrices. Within the CF lung, mucoid *P. aeruginosa* secretes quorum sensing autoinducers (QSAIs) - signalling molecules that regulate virulence factor expression, biofilm proliferation and biofilm maintenance. The nature of how these molecules interact with the EPS is poorly understood, despite the fact that these molecules must pass through EPS material to reach neighbouring sub-populations. For the first time, interactions between two QSAI molecules utilised by mucoid *P. aeruginosa* in the CF lung, C_4-HSL and PQS, and the EPS has been studied at the atomic scale using theoretical modelling techniques, namely, molecular dynamics (MD) and quantum chemical Density-Functional Theory (DFT). A large, exothermic, calcium cross-linked, multi-chain EPS molecular model, representative of the EPS structure observed *in vivo*, was created and MD trajectories were computed to sample modes of interaction between the QSAI molecules and the EPS that occur at physiological equilibrium. The thermodynamic stability of the QSAI-EPS adducts was calculated using DFT. Our theoretical modelling highlights that C_4-HSL has a low propensity to bind to the EPS, whereas the binding propensity for PQS is large - forming thermodynamically stable ionic complexes with EPS-bound Ca^{2+} ions. These simulations provide a thermodynamic rational for the apparent free movement of C_4-HSL, highlight key molecular functionality responsible for EPS binding and, based on its significantly reduced mobility, suggest PQS as a viable target for quorum quenching.

A114

Using bacterial cross-talk to treat infections: Screening and identification of lactic acid bacteria with anti-virulence activity against *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a Gram-positive and opportunistic pathogen known as one of the leading causes of hospital or community-acquired infections that range from mild skin infections to endocarditis and septicemia. The expression of the majority of *S. aureus* virulence factors is regulated by the accessory gene regulator (*agr*) quorum-sensing system. The bacterial communication is based on a two-component signal transduction system, agrAC, which responds to auto-inducing peptides (AIPs), encoded and secreted by AgrBD. Other staphylococci produce auto-inducing peptides able to inactivate *S. aureus* quorum sensing system, suggesting *agr* is an inter-species communication system. We hypothesize that probiotic bacteria reported to improve treatment of *S. aureus* infections are able to do it through the repression of the staphylococcal quorum-sensing system. We screened a collection of lactic acid bacteria against *S. aureus* using quorum sensing transcriptional reporters and identified strains with antibacterial and anti-virulence activity, i.e. quorum sensing inhibiting activity. Cell-free supernatants from several strains of lactic acid bacteria showed inhibition of quorum sensing in *S. aureus*, especially *Lactiplantibacillus plantarum*. On the other hand, cell-free supernatants from *Carnobacterium* spp. strains inhibited *S. aureus* growth. Synthetic auto-inducing peptides from *L. plantarum* were also investigated and were found to inhibit quorum sensing without affecting staphylococcal growth. Lactic acid bacteria, including known probiotics, are promising candidates to develop anti-virulence therapies against *S. aureus* infections.

A116

Developing anti-viral small molecules using a novel Affimer-guided approach

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Abstract

There is constant need for effective therapeutics to tackle both existing and new viruses. We have generated a novel method of developing anti-viral small molecules whereby the loop structures from Affimers, which are small antibody-like proteins expressed in bacteria that bind to target molecules through two hypervariable loops, act as starting references in a ligand-based approach. For proof of concept we have used two systems; Crimean-Congo Haemorrhagic Fever Virus (CCHFV) and Influenza A Virus (IAV).

CCHFV has a fatality rate of ~30%, no effective therapy and it is ranked in the top 10 highest priority viruses for research by the World Health Organisation. IAV exists as seasonal strains, which are estimated to kill ~500,000 people annually, and rarer pandemic strains which are even more fatal. Both viruses require new treatments to tackle them.

The first approach used a previously determined crystal structure of an Affimer bound to CCFHV nucleoprotein (NP). Small molecule mimics were generated of the Affimer loop involved in the binding and screened in a cell-based assay using a CCHFV mini-genome system, which identified a hit compound with low micromolar potency.

The second approach was based off an Affimer which bound the Haemagglutinin (HA) spike protein from IAV, but for which there was no prior Affimer-bound structure. A hit was identified from these mimics using a cell-based assay and its binding is being validated with cryo-EM structures obtained using a cutting-edge grid preparation device. This work highlights how Affimers and cryo-EM can be applied in small molecule discovery.

A117

Graphene oxide/Silver-based nanocomposites inhibit early-phase lentiviral infection

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Abstract

Rationale: Graphene is the most prominent 2D carbon material that has been extensively explored in biomedical research. Due to antimicrobial properties graphene oxide (GO) represents a material that could potentially limit the spread of pathogenic infections. In this study, we evaluate the antiviral properties of Ag NPs-modified GO-based nanocomposites (NCs). Using HIV-1 lentiviruses (LVs) as a model of enveloped virus we showed that NCs efficiently inhibits the early phase of infection by perturbing the viral entry into the host cell.

Methods: The physio-chemical properties of the synthesized materials have been characterized using various spectroscopic and electron microscopy methods. The anti-viral efficacy of NCs was measured using HeLa and THP-1 cells. The effects of the NCs on the viral particles during the cell entry and the early phase of the infection were studied using fluorescence-microscopy imaging. A probable mechanism of indirect inhibition of infection by the cell-penetrating NCs such as induced immune signaling was studied by qPCR. Cytotoxicity of the NCs was studied using biochemical and imaging approaches.

Results: NCs significantly inhibit pseudoviral infection when incubated with the viral particles prior to infection. Time-dependent assays and fluorescence imaging methods point towards a mechanism of inhibition of viral entry through physical interaction with pseudoviral particles. Interestingly NCs showed an even stronger antiviral effect when introduced several hours post-infection.

Discussion: Presented results show that carbon-based materials play an important role in the inhibition of microbial infections and provide a potential option as an anti-microbial material in future medicine and the engineering of medical instruments.

A118

Using yeast as an oral delivery system of vaccines and other biological products

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Abstract

The development of innovative oral delivery systems using mutant yeast cells to release the active compounds in the human digestive environment has been considered. This provides several advantages such as protecting the active compounds against the digestive conditions and regulation of gene expression and protein release.

Strains of Saccharomyces cerevisiae capable of lysis upon conditional down-regulation of cell-wall biogenesis genes (SRB1 and PKC1) have been reported showing that they lyse and release recombinant protein not only under laboratory conditions, but (more importantly) under conditions found in the human stomach and duodenum. These findings provide such conditional lysis strains could be used as an integral part of a system for the oral delivery of therapeutic proteins.

However, the current mechanism of conditional lysis is based on the use of the MET3 promoter which requires addition of methionine and cysteine for down-regulation of SRB1 and PKC1. This requirement makes it difficult to apply in vivo.

In this study, we reasoned those promoters, suitable for in vivo down-regulation of lysis inducing genes, could be identified amongst yeast genes whose transcript abundance is reduced under conditions found in the human gut. A microarray experiment identified a number of candidate genes with significantly reduced transcript levels under simulated human gut conditions. The greatest effects were seen with ANB1, TIR1, and MF(ALPHA)2), and we propose that their promoters have the potential to be used in vivo to achieve yeast lysis in the gut.

A120

Decolonising the Life Science Curricula at the University of Glasgow

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Abstract

Movements such as the Rhodes Must Fall and Black Lives Matter have highlighted the need for 'decolonising the curriculum' efforts in academia. Decolonising the curriculum seeks to acknowledge and dismantle the way that imperialism and colonialism have shaped global education, with a focus on inclusion and visibility of a wider range of viewpoints that have been historically excluded. To create awareness of the Decolonising the Curriculum movement and its relevance to Life Sciences, tutorials were developed by students undertaking their final year projects and delivered to undergraduate students in the Microbiology and Immunology programmes at the University of Glasgow. The tutorials acted as an introduction to the movement, focusing on contextualising how the material linked to existing topics within their respective curricula, through group discussions and case studies. Knowledge progression, as well as the resources produced, were evaluated by pre and post-questionnaires. Throughout the tutorial, students were receptive to and engaged with the subject material, taking active roles in group discussions. General feedback from students was largely positive with clear indications of knowledge progression. Thus, highlighting a desire for and value in the incorporation of 'Decolonising the Curriculum' and other 'Equality, Diversity, Inclusion' material within their curricula. With ever-growing pressures to address issues of inequality and colonial histories, universities in the UK are slowly beginning to work with students and take action. Our work showcases the relevance and scope of these issues within Life Sciences subjects

A121

Translating personal experiences and lessons learned through virtual primary school outreach programmes into Microbiology Teaching in Higher Education

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Abstract

Virtual platforms which support teaching and learning in higher education have taken a central place in our higher education settings. This has become even more apparent during the global SARS-CoV2 (Covid-19) pandemic, which forced a transition to purely online/virtual teaching and learning and more recently to a blended teaching and learning approach. Pandemic restrictions also impacted on face-to-face schools and community outreach programmes. Here, I reflect on personal experiences on how the dynamics brought about by the pandemic shaped my schools outreach initiatives, and share lessons we can draw from this to better support teaching and learning and harness virtual online platforms for enhanced teacher and student experience in Higher education.

A122

A rapid practical class for teaching the principles of selective and differential culture

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Abstract

The pandemic has severely impacted on face to face teaching. At our institution we were keen to deliver Microbiology practical teaching through 2020 and 2021 under strict social distancing. This was facilitated by reducing the capacity of teaching laboratories and limiting the duration of in person teaching.

With reduced contact time we sought to deliver practical content that might expeditiously acquaint the students with standard techniques in bacteriology, i.e. pure culture, selective and differential media, and microscopy. Students were required to attend two practical sessions of 75 minutes duration. Prior to attending the practical class students had to undertake video instruction on Microbiological techniques using a suite of in-house produced videos “Videos in Microbiology”. These videos were logically structured within an Articulate environment to provide for a pleasing learning environment.

To teach pure culture, students were provided with a mixed plate culture of *Escherichia coli* and *Staphylococcus aureus.* To facilitate differentiation of these species X-GAL was incorporated into the media, this led to easily discernible colonies that were either yellow or blue. Students sub-cultured onto Blood Agar, Mannitol Salt agar, and MacConkey agar on Day 1. On Day 2 competency at streak plating was judged. Students then performed Gram’s stains on isolated colonies and were again judged competent on their ability to stain a smear and visualize bacteria microscopically.

In contrast to the pre pandemic era, students worked singly and in labs with much reduced occupancy. This, combined with the approaches outlined above, led to better student learning outcomes.

A123

Heterologous expression, purification, and immunomodulation of the recombinant lipoprotein GUDIV-103 from *Ureaplasma diversum*

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Abstract

Ureaplasma diversum infects cattle and can cause inflammatory process in the genital and reproductive systems. Membrane-associated lipoproteins (LAMPs) are considered the main virulence factors of these bacteria. The objectives of this study were to express and purify *the U. diversum* recombinant lipoprotein GUDIV-103 and evaluating the immunological reactivity and immunomodulation of this protein in bovine cells. Plasmids containing the *gudiv-103* sequence were overexpressed in *Escherichia coli*. GUDIV-103 was purified and inoculated into rabbits for polyclonal antibody production followed by indirect ELISA and Dot blotting tests to assess the kinetics of antibody production. For the expression of this lipoprotein in field isolates was performed Western blotting using anti-rGUDIV-103 serum and proteins from 42 strains of *U. diversum*. The mitogenic potential was evaluated in flow cytometry through the lymphoproliferation assay in bovine peripheral blood mononuclear cells (PBMC). Expression analysis of cytokines, iNOS, and caspase-3 genes was performed by qPCR. NO and H_2O_2 dosage was performed in PBMC supernatant culture. ELISA and dot blotting indicated that rGUDIV-103 induces high production of specific antibodies in rabbits. Antibodies produced against the ATCC 49783 strain were also able to recognize rGUDIV-103. Different concentrations of rGUDIV-103 induced higher gene expression of IL-1β, TNF-α, TLR2, TLR4, iNOS, and caspase-3 in PBMC ($p < 0.05$) when compared to untreated cells. Furthermore, the recombinant lipoprotein induced the lymphocyte proliferation. Thus, rGUDIV-103 has antigenic and immunogenic properties, being able to modulate the immune system induced the expression of pro-inflammatory cytokines and lymphoproliferation, being, therefore, a promising protein for immunobiological applications.

A124

Candida albicans biofilm in suicidal protection of *Porphyromonas gingivalis*

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Abstract

Mixed biofilms formed by bacteria and fungi allow microorganisms to create environments that ensure their survival in adverse conditions and provide metabolic support but also regulate mutual population size or fungal cell morphology.

In recent years, it has been identified that Candida albicans – the yeast involved in mucosa colonization had also an increased frequency of occurrence in subgingival mixed-species biofilm isolates from patients suffering from periodontitis. The dual-species biofilm, formed between C. albicans and Porphyromonas gingivalis is more pathogenic than single-species biofilm.

To determine the reasons for these changes, we performed MS analysis of the mixed biofilm matrix. It indicated a significant increase of matrix proteinous components, involved in yeast cell metabolism, which may have an additional adhesive function on the yeast cell surface. Mutual contact of both pathogens within the biofilm structure also increased the expression of genes encoding typical yeast adhesins (Hwp1, Als3, Eap1) and genes involved in the production of biofilm matrix components and regulation of yeast cell filamentation. On the other hand, Inhibition of yeast mannan synthesis resulted in the decrease of bacterial growth within the mixed biofilm, confirming the roles of mannans or mannoproteins in bacterial cell adhesion and protection.

Observed changes were accompanied by increased consumption of oxygen by yeast cells located within the mixed biofilm, which was conducive to increased bacterial viability and bacterial resistance to antibiotic treatment.

This work was financially supported by the National Science Centre of Poland (grant no. 2019/33/B/NZ6/02284 awarded to MR-K).

A125

The influence of iron regime on the human gut microflora – an in vitro batch culture approach

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Abstract

Iron is an essential micronutrient for nearly all living organisms, including microbes. Iron-deficiency related anaemia (IDA) is a major public health problem that affects more than 2 billion people globally and this represents 24.8% of the world's population. IDA is treated by iron oral supplements; however, iron supplementations have been reported in different intervention studies to influence the composition of the gut microbiota causing a decrease in the level of commensal bacteria and supporting the growth of pathogenic microbes. According to World Health Organisation (WHO), iron deficiency is the most common cause of anaemia worldwide, with infants, children and women at higher risk, making it a global public health problem. Much of iron deficiency is as a result of poor iron dietary absorption since iron is poorly absorbed (~15%). Absorption inhibition factors (iron chelators) like phytate and tannins, have been reported to act as potent iron uptake inhibitors.

The aim of my research is investigating the influence of different iron sources as well as dietary components like tannin and phytate on gut microbiota composition and metabolic activity. This aim is being progressed using *in vitro* anaerobic mini-batch cultures under a range of iron regimes, inoculated with human gut microbiota. Our preliminary findings show that the absence of iron and haem is associated with a decrease in the abundance of Enterobacteriaceae whereas their presence causes a significant shift in the gut microbiota composition and metabolic activity.

A126

Changes in gut microbiome in the course of prostate cancer in mice

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Abstract

Prostate cancer (PCa) is one of the most common cancers among men in the world, therefore attempts are being made to understand the pathomechanism and course of this disease, incl. data on PCa and GI microbiome. Here the factors disturbing the bacterial homeostasis were analysed by the chemical markers ie. short-chain fatty acids (SCFA) and 3-hydroxy fatty acids, markers of lipopolysaccharide to assess the inflammatory potential of gut microbiota in course of PCa.

The levels of SCFAs and 3-OHFAs in stool of mice with induced PCa (TRAMP-C1 or TRAMP-C2 cells) have been determined to reveal the intestinal microbiota homeostasis disorders related to a disease progression. An analytical approach: HPLC with UV/Vis and GC-MSMS provided the laboratory platform to follow the microbiome changes in course of PCa.

The most apparent changes were recorded on days 21-40 of the experiment, when the growth of neoplastic tumors was the highest. A decrease in the number of *Pseudomondales*, *Bacteroides* and *Fusobacteriales* in the stool samples for the prostate tumor model group compared to the control were recorded.

Around days 21-40, the animals developed neoplastic tumors of the prostate at a very vigorous pace, likely leading to an increase of butyric acid and acetic acid in mice with TRAMP-C1 (day 21) and TRAMP-C2 (day 40).

The analysis of SCFA and chemical LPS markers provided a quick, inexpensive tool for microbiome assessment, complementary to DNA sequencing, offering an interesting target for research on microbes-host-cancer interaction.

A127

An impact of a high-fiber diet on the intestinal microbiome in the course of colorectal cancer

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Abstract

The accurate composition and metabolism of the intestinal microbiome to the greatest extend depend on the diet. An adequate intake of dietary fiber results in a large production of short-chain fatty acids (SCFA), contributing to the anti-inflammatory reaction by participation in maintaining a balance between the suppression of inflammatory mediators (TNFα, IL1β, IL6) and the induction of anti-inflammatory cytokine (IL10).

Here we have determined the levels of SCFAs in stool of mice with colorectal cancer and a control group to assess microbiota homeostasis disorders related to a cancer progression, as well as an influence of high-fiber diet (cellulose or potato starch) on the concentration of SCFA in feces and cancer progression.

Analytical methods (HPLC with UV/Vis) and NGS sequencing were applied.

The diet was the main modulator of the gut microbiome: a diet rich in cellulose did not increase the fecal SCFA concentration except of lactic acid. Moreover, a diet rich in potato starch significantly changed the SCFAs profile and increased their fecal concentration.

A diet enriched with cellulose had a positive effect on an intestinal abundance of *Akkermansia muciniphila* in cancer-affected animals. *Lactobacillus* abundance decreased when diet enriched by 20% cellulose was used. Animals consuming cellulose-rich diet reveal a lower *Firmicutes*:*Bacteroidetes* ratio comparing to group on standard diet.

A diet with potato starch (type 2 resistant starch) caused an increase of *Bifidobacterium* and *Faecalibaculum*, and a decline in abundance of *Blautia*, *Peptocdoccus*, *Ruminococcus* UCG-010 and *Anaeroplasma*.

A128

Impact of cyclosporin A treatment on the host microbiome, immune response and disease severity in the DSS model of colitis

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Abstract

The pathogenesis of colitis is complex, with interaction between the immune system, epithelia and microbiome contributing to inflammation. Cyclosporin A (CSA) is an immunosuppressant used to treat refractory colitis. We investigated changes in the disease profile in a DSS (Dextran sulphate sodium) induced colitis mouse model with or without treatment with CSA to examine the relationship between immunosuppression, resolution of dysbiosis and disease.

Mice given continual access to 5% DSS solution developed colitis, showing increased bodyweight loss and stool scores. Those treated with CSA showed an in increase in bodyweight loss until D6 with less bodyweight loss at D7 than DSS only mice. These mice also had decreased stool scores suggesting an ameliorative impact of treatment. Faecal samples were taken on D0, D3 and D7 to determine the composition of the microbiota. To determine the effect on tissue specific immunity, colon explants were cultured for 48h +/- LPS stimulation. Restimulation of explants with LPS revealed that treatment with CSA supresses the immune response; IL-6, IL-1b, MCP-1 and TNFa were all statistically increased in colons from DSS treated compared to naïve and CSA treated animals.

In this model of DSS induced colitis we were able to show the differing cytokine profiles of distal colon explants following stimulation with LPS. Treatment with CSA resulted in an altered disease profile that may suggest suppression of immune reaction to the bacterial component of the microbiota. Analysis of 16s rRNA data will allow us to determine if this is linked to resolution of dysbiosis.

A129

Lacticaseibacillus rhamnosus GG lysates inhibit *Staphylococcus aureus* growth and enhance the expression of epidermal barrier proteins in organ cultured human skin

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Abstract

The incidence of antibiotic resistant strains of *Staphylococcus aureus* makes the requirement for innovative approaches to prevent infection critical. We have investigated the effect lysates of *Lacticaseibacillus rhamnosus* GG (LGG) have on *S. aureus* growth on human skin in organ culture. *S. aureus* multiplied on human skin reaching a viable count of $3.5x10^9 \pm 9x10^8$ CFU after 24 hours of culture. However, a lesser bacterial count of $4x10^7 \pm 2x10^6$ CFU was recovered in the presence of 0.2 µg/mm^2 of LGG lysate. FISH experiments targeting *S. aureus*, inicated that treatment with LGG lysate prevented the pathogen penetration into human skin. Furthermore, topical application of LGG lysate to organ cultured skin resulted in a concentration-dependent increase in the expression of key skin barrier proteins and also in the expression of specific antimicrobial peptides.

These data suggest that topical application of a probiotic lysate could potentially inhibit the colonization of skin by *S. aureus*. LGG lysate improves the physical and antimicrobial barrier of the epidermis, therefore enhancing the skin's immune defence against invading microorganisms, which may help in prevention of infection.

A130

A combination of identification techniques reveals the dynamics of mother-to-infant microbial transfer.

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Abstract

An infant is considered to inherit a significant proportion of its microbiota from its mother during birth. This begins a lifelong and dynamic relationship with microbes that has enduring implications for host health. Several studies have shown that maternal and environmental factors influence the establishment of the infant gut microbiome. For example, the gut microbiome of Caesarean born infants is distinct to that of vaginally delivered infants. Using amplicon sequencing there is growing evidence that related mothers and new-borns are more likely to share similar species from taxa including *Bacteroides*, *Bifidobacterium*, and *Escherichia*. Furthermore, shotgun metagenomics has revealed a strain level heritability of specific bacteria. The requirement to call a bacterial strain with sufficient confidence, however, is limited by both sequencing read depth and coverage. Moreover, metagenomic sequencing may not detect transfer of strains that are present in very low abundance relative to the entire microbiota. We attempted to address these issues during an investigation of a cohort of pregnant women from 16-weeks gestation through to 1-month post-partum (including infant sampling). From the samples collected, we used a combined identification approach to determine the extent of maternal-infant (dyad) strain transfer. We additionally collected detailed perinatal metadata to elucidate factors that influence this transmission. Strain transmission was detected in more than 50% of dyads, with a strong impact of delivery mode on this occurrence.

A131

How do hybrid two-component systems (HTCS) in gut bacteria regulate global gene expression?

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Abstract

The human gut is home to microorganisms that have formed a symbiotic relationship with their hosts to create the human gut microbiota (HGM). A key metabolic function of the bacterial genus *Bacteroides* in the HGM is the ability to break down host and dietary polysaccharides which cannot be broken down in the host, due to polysaccharide complexity and the lack of adequate enzymes. *Bacteroides* utilise novel hybrid two-component systems (HTCS) to sense specific oligosaccharides and regulate gene expression for various proteins needed for the catabolic process. The histidine kinase (HK) and response regulator (RR) are the two main functional components of the HTCS which are linked together in one polypeptide that spans the cytoplasmic membrane of the bacterium.

Electromobility Shift Assays (EMSAs) have confirmed that the HTCS BT3786 (specific to yeast α-mannan) binds DNA at two out of four predicted HTCS binding sites. Protein-DNA interactions will be investigated further through RR phosphorylation and mutation experiments. Having successfully validated the introduction of 3xFLAG tags in the N-terminus of the HTCS BT3786 *in vivo,* we hope to perform Chromatin Immunoprecipitation-Sequencing (ChIP-seq), to confirm which proteins are regulated by BT3786, during yeast α-mannan digestion. Additionally, the Green Fluorescent Protein (GFP) labelling technique may enable us to microscopically visualise the localisation of the DNA in the *Bacteroides* cell, during polysaccharide degradation.

Understanding how this crucial system is modulated at the cytoplasmic membrane, will be advantageous in future disease treatment plans, that require specific bacterial colonisation into patients to restore essential metabolic processes.

A132

Diversity and distribution of the tryptophanase gene among gut bacteria

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Abstract

Indole is the central metabolite produced from tryptophan by gut bacteria, through the action of the enzyme tryptophanase. Tryptophanase catalyzes the conversion of L-tryptophan to indole and ammonium pyruvate and is encoded by the *tnaA* gene. Numerous studies have shown that the microbiota-derived indoles play a significant role in bacterial intercellular signalling and also impact upon cardiovascular, metabolic, and psychiatric diseases. However, which gut microbes in the microbiome have *tnaA*, and therefore potentially produce indole, remains largely unknown. Here we explore the genetic diversity of *tnaA* in its most well-characterised bacterium, *E. coli*, and within the gut microbiota itself (Unified Human Gastrointestinal Genome (UHGG) collection). We found that three of phyla accounted for >90% of the 470 TnaA identified. Bacteriodiota and Firmicutes, two of the dominant phyla in the gut microbiome, could be significant contributors of indole production in the gut. Further, the maintenance of *tnaA* in clusters in the gut environment suggests the importance of tryptophanase to individual species, and to the microbiota. The conclusions obtained in this study give insight into signalling in the microbiome, as well as the potential for indoles to be used as therapeutics in designing the gut environment.

A133

A meta-analysis of 16S rRNA studies of the early rumen microbiome and its malleability

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Abstract

Like most gut microbiotas examined to date, metataxonomic studies have identified the distinct patterns of colonisation in the rumen in early-life. However, differing results have been obtained between studies, including differing hypotheses regarding the timing of stages of microbial colonisation, and the microbes relevant these stages. Additionally, whilst there have been studies highlighting variation in adult rumen microbiota compositions, whether importance of early life in establishing the adult microbiome and which (if any) microbes can be manipulated to *in vivo* in early life to achieve a desired microbial composition in the adult.

This study conducted a re-analysis of available 16S bacterial rRNA rumen microbiota data spanning the first one hundred days of life. The goal was to identify shared taxonomy at different stages of rumen development and identify the patterns of colonisation. Sequencing data from thirteen domestic ruminant studies representing 1391 samples was obtained and a standardized pipeline was used to re-analyse all the data utilising Kraken 2/Bracken. This was followed by individual analysis of each of these to understand the changes in community structure during early life.

Consistent patterns of colonisation were observed for microbes in early life even across different ruminant hosts and feeding strategies. For instance, Proteobacteria and Fusobacteria were consistently observed to decrease over dietary stage in early life and Actinobacteria which consistently increase. However, interestingly several phyla, including two of the most well studied (Firmicutes and Bacteroidetes) showed no consistent colonisation trends across the studies, suggesting that they may be manipulable in early life.

A135

Hospital wastewater pipes as a reservoir of extended-spectrum β-lactamase producing bacteria and antimicrobial resistance genes

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Abstract

Hospital acquired infections caused by extended-spectrum β-lactamase (ESBL) producing bacteria have become a significant global healthcare issue in recent years. These infections can be transmitted by direct human contact or via the clinical environment, such as wastewater pipes. In this study, we focused on the wastewater system within a large teaching hospital to investigate its potential as a reservoir of ESBL-producing bacteria and the antimicrobial resistome contained therein. Twenty pipe sections from showers, toilets and sinks were analyzed using culture-based approaches, fosmid library generation and metagenomic sequencing, followed by antimicrobial susceptibility testing against a number of commonly used antibiotics. Culture-based approaches, using ESBL-selective media, yielded eleven species of ESBL-producing bacteria with a high degree of antibiotic resistance. Metagenomic sequencing indicated diverse microbial populations and the widespread presence of antimicrobial resistance (AMR) genes. Fosmid library construction resulted in the cultivation of amoxicillin, cephalexin and meropenem resistant clones, with sequenced fosmids bearing AMR genes corresponding to the phenotypes observed. This study represents a wide-ranging and sophisticated analysis of a clinical wastewater system, both in terms of microbial populations and antimicrobial resistomes present. Antimicrobial susceptibility testing determined the presence of a number of multi-drug resistant species, confirming the genotypes revealed by environmental DNA sequencing. This study demonstrates the potential of clinical wastewater systems to act as reservoirs of AMR genes and ESBL-producing bacteria. Ongoing work in this area may help to inform infection prevention and control policies within healthcare settings, with a view to reducing the incidence of infections caused by ESBL-producing bacteria.

A136

An *In Vitro* Approach: The Effect of Iron and Lactoferrin on the Infant Gut Microbiota

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Abstract

The global prevalence of anaemia in children of 6 to 59 months is 42% according to World Health Organisation. While iron supplementation is widely used as a treatment, numerous studies reported the adverse effects of high doses of iron such as increased pathogenesis in the gut microbiota. With emerging evidence of the prebiotic and antibacterial (especially for pathogenic species) effect of lactoferrin, an iron-binding protein naturally high in human breastmilk, we aim to explore how different iron regimens affect the infant gut microbiota and how lactoferrin might influence such effects.

Different iron and lactoferrin regimes that are equivalent to those provided by widely available formula milk and human breastmilk were applied in *in vitro* batch fermentation models, inoculated with infant gut microbiota (n=3). The microbial composition and short-chain fatty acids (SCFAs), the metabolites from gut microbiota, were measured during the fermentation that was performed under conditions designed to be similar to those of the infant large intestine for 48 hours.

The main phyla of infant gut microbiota were Proteobacteria, Firmicutes, and Actinobacteria. At the family level, insignificant but notable trends in *Lactobacillaceae* (increase with lactoferrin) and *Bifidobacteriaceae* (increase with iron) were observed. The alpha diversity was much higher with the conditions with a high dose of lactoferrin, but no significance was found. As for SCFA, a greater increase in acetate and lactate was resulted by lactoferrin containing treatments.

The outcomes so far suggest a possible effect of lactoferrin on improving microbial diversity and promoting SCFA-producing bacteria.

A137

Interpretable machine learning model to predict the AMR phenotype of bacteria from genomic data

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Abstract

The microbiome harbours numerous antimicrobial resistance (AMR) genes. Yet, our understanding of AMR's role within the microbiome when antibiotics are absent, is unclear. AMR phenotype can be distinguished using routine laboratory-based methods. However, numerous species within the microbiome are difficult to culture. Therefore, there is a limit to what can be achieved using culture-based methods. Using computational approaches to predict the AMR genotype in genomic data is efficient; however, how this relates AMR phenotype of the organism in still an open question.

We used an interpretable decision tree machine learning algorithm to examine the ability of computationally predicted AMR gene profiles to accurately predict the host AMR phenotype using 16,664 genomes with MIC profiles from PATRIC.

We found that using training sets of antibiotic-specific subsets of AMR genes was less accurate at predicting AMR phenotype than using all AMR genes, regardless of the antibiotic to which they provided resistance. This suggests that some AMR genes may provide resistance to a wider range of antibiotics than previously thought. Furthermore, the taxonomy of the training set played a large role in the accuracy of the resulting AMR phenotypic predictions highlighting the importance of deriving AMR phenotypes from a wider range of species than the human pathogens dominating the current databases.

To facilitate this kind of large-scale analysis we created a novel tool which automates the process of creating input files for WEKA: https://github.com/lucy2611/CSV_2_arff.

Future work will investigate the effect of including non-AMR related genes on the ability to predict AMR phenotype.

A138

In-depth insights into cervicovaginal microbial communities and hrHPV infections using high-resolution microbiome profiling

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Abstract

The cervicovaginal microbiome (CVM) correlates with women’s cervical health, and variations in its structure are associated with high-risk human papillomavirus (hrHPV)-induced high-grade cervical lesions. The CVM exhibits five community state types (CSTs) based on microbial dominance and composition; however, elucidating the impact of CSTs in health and disease is challenging because current sequencing technologies have limited confident discrimination between bacterial species that shape microbial communities. This study aimed to apply high-resolution microbiome profiling to obtain in-depth and unambiguous insights into the composition of the CVM. Circular probe-based RNA sequencing (ciRNAseq) was used to profile the CVM of a cohort of healthy women from the Dutch population-based screening program (n = 343) and a second cohort of hrHPV positive women with known clinical outcomes (n = 300). CSTs were established and correlated to clinical outcomes. Here we confirmed the recently defined subgroups of CSTs I, III, and IV, which display dominance by L. crispatus, L. iners, and high diversity, respectively, in the CVM. We discovered that these microbial states strictly depend on the bacterial species L. acidophilus, which is present in CSTs I-B and III-B but absent in I-A and III-A, and M. genomosp type 1, which is present within CST IV-B and absent in IV-A. Notably, CST I-A associated with uninfected conditions, while CST IV-A with hrHPV infections and cervical disease. In conclusion, we corroborated and characterized new subdivisions of cervicovaginal CSTs, which may further advance our understanding of women's cervical health and hrHPV-related progression to disease.

A139

Using soil microbiota to suppress plant pathogen colonization in hydroponic systems

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Abstract

Commercial hydroponic greenhouses are important for tomato production, as they allow for more efficient water, fertiliser, and pesticide usage than traditional soil-based systems. However, crops grown in soilless systems are often more susceptible to disease.

This research aimed to characterise the mechanisms of plant protection by disease-suppressive soils and investigate whether this beneficial activity can be transferred to hydroponically grown plants. To identify a suitable suppressive soil, pre-germinated tomato seeds (cv. Moneymaker) were grown in six well-characterised field soils with known crop histories and land management practices. After two weeks of growth, the shoots were challenged with the hemibiotrophic bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* DC3000, which expresses the *Photorhabdus luminescens lux* operon (*Pst*-LUX). Leaf colonisation was scored 2 days later by quantifying bioluminescence intensity from infected leaves. The leaves of plants grown in one soil displayed statistically lower levels of *Pst*-LUX colonisation, which was not routinely associated with reduced growth, indicating induced systemic resistance (ISR) by non-pathogenic soil microbes.

To confirm that the observed disease-suppression can indeed be attributed to microbial activity and ISR, a microbial fraction from the selected soil was transplanted to pre-germinated tomato seeds in rockwool and assessed for systemic resistance against *Pst*-LUX. Leaves from hydroponically grown plants treated with the microbial fraction displayed reduced *Pst* colonisation compared to non-treated controls. Dilution or sterilisation of the microbial fraction prevented the resistance, indicating a microbial basis. Together, our results show that microbial activity in the disease suppressive soil mediates ISR in tomato, which can be transferred to hydroponic growth medium.

A140

Effect of supplementation with low-moisture, sugarcane molasses-based block on changes in the rumen microbial relative abundance on steers fed low quality forage

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Abstract

We evaluated the effects of supplementation with low-moisture, sugarcane molasses-based block (LMB) on steers fed low-quality forage on changes in the rumen microbial relative abundance through 16S rRNA gene sequencing, generated on the Illumina Miseq. Six rumen cannulated Nellore steers (23 months, 350 ± 10 kg) were distributed in a 3 × 3 double Latin square design. The treatments were composed of Urochloa brizantha (Hochst.) Stapf, "Marandu" hay as an exclusive source of bulks (93.65% DM, 3.97% CP and 81.76% NDF) and supplements: mineral blend with urea (UR, [urea, salt, mineral-vitamin premix]), a commercial protein supplement (PS, [corn grain, soybean meal, urea, salt, and mineral- vitamin premix]) or protein block based on low-moisture cooked sugarcane molasses (LMB, [cane molasses, cottonseed meal, soybean oil, urea, salt, and mineral-vitamin premix]). The LMB supplementation allowed a higher relative abundance of *Quinella.spp* (P = 0.033), *Moryella.spp* (P = 0.011), and *Marvinbryantia.spp* (P = 0.041) population when compared to UR. Also resulted in the lower ruminal relative abundance of *CPla-4 termite* group (P = 0.011) and *Rikenellaceae.RC9 gut group* (P=0.033). Steers supplemented with PS had a greater ruminal relative abundance of *Mogibacterium.spp* (P = 0.006), *Ruminococcaceae UCG-004* (P = 0.016), and *Papilibacter.spp* (P = 0.037) when compared to UR. The *Clostridium sp.CAG-352* (P = 0.054), *Lachnoclostridium 10* (P = 0.054), *Prevotellaceae UCG-004, Ruminococcaceae-UCG-011*, and *Succinivibrio.spp* only were identified in the rumen of steers fed PS. Steers supplemented with UR showed a higher ruminal relative abundance of *Fretibacterium.spp* (P = 0.009), and *Lachnospiraceae ND3007 group* (P = 0.024).

A141

Novel fully primary human airway epithelium-alveolar macrophages in vitro co-cultures models to study host pathogen interactions

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Abstract

Being the first line of defense of the organism against airborne pathogens like bacteria and viruses, the respiratory epithelium acts as a physical barrier as well as an efficiency mucociliary escalator. Furthermore, the airway epithelium is also a potent immune-regulator which orchestrates both innate and adaptive immune responses upon bacterial or viral infections.

Many animal models have been used to study lung infections, but the relevance and predictability of animal models are still questionable. Here we established a new co-culture model using well characterized, standardized human airway epithelium such as MucilAir™, SmallAir™ and human lung macrophages ($CD45^+$,$HLA\text{-}DR^+$, $CD206^+$, $CD11b^+$and $CD14^-$) for studying bacterial and viral infections. The alveolar macrophages were not only able to adhere to the epithelial cells, but also functional: The macrophages were capable of phagocytosis, evaluated using pHrodo™ Red (S cerevisiae Bio-particles Conjugate). Moreover, the co-culture models respond to pro-inflammatory stimuli such as LPS, TNF-a and Poly(I:C) with an increased IL-8 secretion.

Upon bacterial infection with methicillin-susceptible *Staphylococcus aureus* strain (MSSA), compared to MucilAir™ monocultures, MucilAir™-macrophages showed stronger immune responses: (i) a reduction of bacterial growth (up to 1.5Log10 CFU) and (ii) decreased upregulation of IL-8 and b-defensin-2 secretions. Interestingly, greater difference was observed for *Streptococcus pneumonia* (Sp19F): The presence of macrophages led to a decrease of 3.5Log10 CFU after 24 hours of culture (N=12) versus MucilAir™ alone.

These novel *in vitro* models might find applications in understanding the role of immune-epithelial cell interactions in infection diseases.

A142

Exploration of trimethylamine *N*-oxide metabolism in human gut bacteria

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Abstract

Trimethylamine *N*-oxide (TMAO) is a compound found in fish and other foods. Gut bacteria convert TMAO to trimethylamine, which is taken up by the liver and converted back to TMAO by hepatic enzymes. The protein TorA, predicted to be encoded by *Escherichia* and *Klebsiella* spp., was reported to drive TMAO to TMA metabolism in the human gut, but preliminary data indicated this not to be the case. Further work aimed to explore non-Tor proteins in human gut-associated bacteria, with further characterisation of a caecal isolate of *Klebsiella pneumoniae* as a model of TMAO metabolism. Bioinformatic analyses determined the prevalence of TMAO metabolism-related proteins in the genomes of ~36,000 human gut-associated bacteria, as well the *K. pneumoniae* isolate. Proteins previously thought to be the most important for TMAO metabolism in the gut were not as common as expected. Work examining the effects of TMAO on the metabolism of *K. pneumoniae* included measuring growth rate, qPCR on anaerobic respiration genes, GC-MS quantifying TMAO-related metabolites and comparison of cell sizes. As a result of the bioinformatic work the protein BisC was identified as potentially being able to reduce TMAO in *K. pneumoniae*. This protein has been expressed in *E. coli* and purified for use in a benzyl viologen assay to determine whether BisC is able to reduce TMAO *in vitro*. By examining TMAO metabolism in gut bacteria more knowledge can be gained on how these bacteria could affect TMAO levels in humans, which could have implications in several different areas of human health.

A143

Re-introduction of microbiota early in life of dysbiotic IL-10 KO mice has a long lasting effect on host immunity and disease outcome

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Abstract

The microbiota has profound influence on the host through interactions with the immune system beginning early in host development. These interactions are crucial in understanding diseases where pathogenesis is not fully understood, such as Inflammatory Bowel Disease (IBD). Here, we examined how re-introducing a microbiota to dysbiotic IL-10 KO mice during development impacted outcomes of colitis, host immune responses and microbial functional changes. Pregnant dams were treated with the antibiotic cefoperazone to vertically transmit dysbiosis. Dysbiotic pups received oral gavage of fecal microbiota transplants (FMT) from control mice, at 2, 3, and 8 weeks after birth. After 23 weeks, mice were supplemented with 2.5% DSS to induce colitis if they did not exhibit colitis symptoms. Colon histology, host RNA transcripts, and immune serum markers were used to determine colitis status, host functional changes, and immune status in adult mice. Shotgun metagenomic sequencing of fecal content collected from dams and pups highlighted microbial functions that differed in the FMT mice compared to dysbiotic groups. Histology suggested mice receiving FMT had less colon inflammation and fewer neutrophils than mice with no gavage or gavage control. Cytokine levels for IL-2 and IL-17 showed significant increase in mice with FMT indicating probable immune education. Initial metagenomic data illustrated differing microbial makeups among treatment groups. Akkermansia muciniphila was highly detected in all mice, while Oscillibacter were detected in control and FMT mice. Understanding how early microbial influences pathogenesis of IBD can lead to development of preventatives or therapeutics for treatment of this increasingly prevalent disease.

A144

eGUT: Developing and validating a platform for computational modelling of the gut

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Abstract

Background

Current research reveals numerous links between the gut microbiota and human health. The majority of microbiota research is performed *in vivo* in rodents, which can be costly, requires ethical considerations and may not always be a reliable analogue of human guts.

Methods

We are building an *in silico* platform for gut modelling – eGUT. eGUT can simulate the activities and interactions of microbes in the gut and the mucosa, which can be connected to a 'rest of the body' compartment. Individual-based modelling allows us to investigate how the behaviours of and interactions between individual cells lead to observed phenomena. eGUT is a highly flexible system that allows users to combine different behaviours and traits in different ways and model a wide variety of organisms.

Results

We have carried out various numerical tests to assess the accuracy of eGUT's reaction-diffusion system and used linear discriminant analysis to identify parameter space in which mucus behaves as a sticky, spreading fluid that coats the epithelium. Validation with simple two-species test cases is underway using both HuMiX and SHIME *in vitro* models. Later *in vivo* validation based on published results is also planned.

Outcome

eGUT allows users to model a wide range of species and metabolic activities in the gut environment without requiring extensive understanding of programming languages. It will be open-source and freely available upon release. Ongoing validation, as well as engagement with potential users, will be used to improve the platform. Researchers interested in using eGUT are encouraged to enquire.

A148

An Improved and Extended Multilocus Sequence Typing (MLST) Scheme for the Genus *Streptomyces* Reveals a Complex Taxonomic Structure

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Abstract

Streptomyces species produce over 60% of all clinically-approved bioactive compounds. Continuing discoveries of new natural products suggest that *Streptomyces* genomes are a promising potential source for novel antibiotics. Comparative genomics and pangenomics are powerful tools for inferring genes involved in the synthesis of novel antibiotics from closely related genomic sequences. Current *Streptomyces* taxonomy is contested, making correct application of these approaches more difficult.

MLST is used for genomic classification by comparing internal sequence fragments of multiple loci. The current *Streptomyces* MLST scheme comprises six markers and 236 sequence types (STs; only two new STs were reported since 2016). With the recent increase in sequenced *Streptomyces* we can now ask: (i) what resolution does MLST offer; (ii) does it reveal useful information about the structure of *Streptomyces* taxonomy; and (iii) does the current marker set adequately discriminate between species (or other useful groups), or could we improve it with a different set of markers?

We extended the current scheme to include all available *Streptomyces* genomes, identifying over 600 novel STs. Using average nucleotide identity, we observed that the scheme diverged form taxonomy and nomenclature, and inadequately captures species diversity and phylogeny: (i) multiple species were found to share a single ST; (ii) multiple distinct STs were required to describe some genomic species; and (iii) some named species were split across unconnected groups of STs in the minimum spanning tree.

Here we demonstrate that the extended MLST scheme provides quantitative motivation for reclassification within *Streptomyces*, and an improved marker scheme.

A149

Integrating genomics into the taxonomy of *Geodermatophilaceae*

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Abstract

The integration of genomic information in microbial systematics along with physiological and chemotaxonomic parameters are nowadays compulsory taxonomic criteria for a reliable classification of prokaryotes. The use of *in silico* analysis Laboratory-based DNA-DNA hybridisation methods have routinely been replaced by ANI and *in silico* DNA-DNA analysis. *In silico* analysis for phenotypic traits are now being introduced to replace characteristics traditionally determined in the laboratory with the dual goal of increasing the speed of the description of taxa and the accuracy and consistency of taxonomic reports. With the aim of taxonomically characterise four novel species within the family *Geodermatophilaceae* (Phylum *Actinobacteria*), we carried out phylogenomic analyses to determine their taxonomic status and explore genotype-phenotype relationships with potential taxonomic value.

Our results pointed out that the genus *Blastococcus* is not monophyletic and a novel genus within the family should be proposed. Besides, taxonomic markers, cellular and physiological features, resulted to be heterogeneous within *Blastococcus* and emendations of several species needed. Genome mining revealed potential overlooked phenotypic characteristics in *Geodermatophilaceae* regarding morphology and physiology. In addition, some phenotypic-genotypic correlations with taxonomic value were confirmed suggesting that some could effectively be replaced by *in silico* tests.

A150

Phylogenomics and the early evolution of Bacteria

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Abstract

Evolutionary trees provide a framework for interpreting biodiversity, with rooted trees particularly important for distinguishing ancestral and derived characters. Bacteria are among the most abundant and diverse cellular lifeforms, but the early history of the domain remains uncertain. This is due both to the immense time spans involved but also the cumulative impact of gene transfer, which can scramble the signal for deep relationships and challenges the concept of a rooted phylogeny as an adequate description of bacterial evolution. Here, I will present some recent and new work that brings new phylogenetic and comparative genomic approaches to bear on resolving the phylogeny and root of Bacteria, the nature of the last bacterial common ancestor, the contributions of vertical and horizontal transmission to bacterial evolution, and the geological timescale of bacterial diversification. Briefly, gene tree-species tree reconciliation and several other "outgroup-free" rooting approaches suggest that the deepest divide within Bacteria lies between the Gracilicutes and the Terrabacteria; that the CPR/Patescibacteria are derived from within Terrabacteria; and that the last bacterial common ancestor was a free-living, rod-shaped cell with a double membrane. Despite a profound cumulative impact of gene transfer during evolution across most gene families and all lineages, the majority of detected gene transmissions are vertical, and a rooted tree provides an apt summary of an important component of bacterial evolution.

A151

Developing ppGpp Target Capture Compounds to Map Stress Response Signalling Networks in *Staphylococcus aureus*

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Abstract

The alarmones guanosine tetra-phosphate (ppGpp) and guanosine penta-phosphate (pppGpp), collectively termed (p)ppGpp, are universally conserved second messenger nucleotides in prokaryotes. (p)ppGpp controls the "stringent response" a complicated process that occurs in bacteria exposed to external stresses such as fatty acid or amino acid starvation. This response ultimately promotes cell survival through a form of quiescence resulting in increased antimicrobial tolerance and thus persistent/chronic infections. Despite the discovery of these alarmones in *Escherichia coli* in 1969, as well as several of their protein targets in later years, a large number of targets are unknown, and how they facilitate this dormant state is incompletely understood. Here we describe progress towards the synthesis of (p)ppGpp target-protein capture compounds using the chemical synthesis of a biotinylated linker fused to enzymatically produced p(s)pGpp. These compounds are required for mapping the alarmone-protein signalling network and revealing (p)ppGpp binding partners in *Staphylococcus aureus* during various stress conditions. This approach has previously been implemented in *E. coli* to yield a plethora of (p)ppGpp targets. As the protein pathways utilised by *E. coli* are fundamentally different to that of *S. aureus*, applying this method to different species will allow for the identification of new protein targets that could aid in the understanding of this stress response.

A152

Examining the Bile Acid Transforming Capabilities of Bacteria Associated with Bile Acid Diarrhoea

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Abstract

Though often misdiagnosed as irritable bowel syndrome due to similar clinical symptoms, primary bile acid diarrhoea (BAD) is estimated to affect 1% of the UK's population. Primary BAD is idiopathic, however, it is thought that dysfunctionality in the regulation of bile acids results in an excessive build-up of bile acids in the colon of BAD sufferers. Bacteria affect the enterohepatic circulation of bile acids via transformation of primary bile acids into secondary bile acids. Using the current literature, we identified microbial gut species predicted *in silico* to have bile acid transformation capabilities based on the presence of the bsh and bai genes required for deconjugation and, dehydrogenation and dehydroxylation respectively. Pairs of these bacteria were mixed at a 1:1 ratio in an anaerobic chamber at 37°C to mimic conditions similar to the colonic lumen and primary bile acids were added to the culture. Subsequently, analysis of secondary bile acids in the culture by high performance liquid chromatography mass spectrometry will allow analysis of the bile acid transforming capabilities of identified bacteria *in vitro*.

A153

The stringent response is required for long-term bacterial viability

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Abstract

Staphylococcus aureus is a bacterial pathogen that often results in difficult to treat infections due to the acquisition of antibiotic resistance, the formation of persister cells or cells becoming small colony variants (SCV). One mechanism *S. aureus* cells use to aid survival during infection is the stringent response, which is a stress survival pathway that reallocates bacterial resources to shut down growth until conditions improve. Even though small colony variants have been linked to a hyperactive stringent response, here we report on the presence and domination of a population of small colonies in a stringent response-null mutant population. This population arose after 14 days of growth in starving conditions, but was not present when bacteria were grown in rich media. The small colonies repeatedly predominated over the wildtype sized colonies, and the phenotype was stable after an outgrowth. We have subsequently characterized the growth, antibiotic resistance profile and fitness of the starving population compared to the wildtype. Understanding the diversity of genetic and regulatory pathways that pave the way towards small colony formation and persistent infections will help us to prevent the formation of long term infections.

A154

Bacterial commensal-to-pathogen transition in the mosquito gut

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Abstract

The mosquito gut harbours diverse microbes which can modulate the capacity of the host to transmit pathogens like dengue virus or *Plasmodium*. Despite the potential of the microbiota to reduce transmission of vector-borne diseases, the interactions between microbes and the mosquito gut are complex and need to be understood before designing such control tools. One key question is how the host can discriminate between beneficial and pathogenic microbes, keeping the former while killing the latter. For example, *Drosophila* pathogens, unlike commensal bacteria, have a nucleoside hydrolase (NH) that produces uracil which activates the DUOX-dependent immunity of the host. This activation results in the production of reactive-oxygen species (ROS) and a shortened host lifespan. Whether this process occurs in mosquitoes is unknown. Here, we investigate how mosquito symbionts can shift from a commensal to a pathogenic status and the mechanisms that allow the host to distinguish between them. Preliminary results suggest that production of ROS activated by uracil may be different between *Drosophila* and *Aedes aegypti*, unravelling an enzyme only present in mosquitoes and differentially expressed between males and females in response to uracil. Interestingly, uracil also seems to affect blood fed and sugar fed mosquitoes in a different way. On the bacterial side, the NH gene is present in some members of the mosquito microbiome but absent in others. Further experiments will include the generation of gnotobiotic mosquitoes with transgenic bacteria to assess if this gene and uracil production are key elements for the commensal-to-pathogen transition in the mosquito gut.

A155

Keep your friends close and your enemies closer? Pathogenic scab-causing Streptomyces spp. are close relatives of wheat root endophytes

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Abstract

Plants roots interact with a large array of below-ground microorganisms that can be beneficial or harmful[1]. While microbial diversity gradually decreases from bulk soil to the bread wheat plant vicinity (the rhizosphere), *Streptomyces* genus abundance increases until inside the root (the endosphere)[2]. We set out to identify and understand how and why some members of the *Streptomyceteceae* family are so well adapted to that ecological niche. We used Pacbio circular consensus sequencing (CCS) of the 16S rRNAs present in the wheat endosphere to detect the most abundant *Streptomyces* species present inside wheat roots. We identified three major clades that are most closely related to the known endophyte species *S.canus* and, more surprisingly, the pathogenic species *S. scabies* and *S.turgidiscabies*[3]. Representative strains of those species were cultured from roots of field-grown wheat plants and bioassays lead us to conclude that the scabies-like strains either didn't acquire or somehow lost their pathogenicity. We hypothesize that wheat pathogens and endophytes have recently diverged and must share some traits favouring their competitiveness in plant roots. Genome sequencing is ongoing and comparative genomics should help us to pinpoint what are these traits.

[1]Trivedi, Pankaj, et al. "Plant–microbiome interactions: from community assembly to plant health." Nature reviews microbiology 18.11 (2020): 607-621. [2]Prudence, Newitt et al (2021). Soil, senescence and exudate utilisation: Characterisation of the Paragon var. spring bread wheat root microbiome. Environmental Microbiome 16:12. [3]Zhang, Yucheng, and Rosemary Loria. "Emergence of novel pathogenic Streptomyces species by site-specific accretion and cis-mobilization of pathogenicity islands." Molecular Plant-Microbe Interactions 30.1 (2017): 72-82.

A156

Developing biosensors for symbiotic algae in coral reefs

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Abstract

Dinoflagellates are a highly diverse group of phytoplankton with considerable ecological impact, including toxic “red tides” and as essential symbionts for coral reefs. Most corals are particularly sensitive to rises in ambient sea temperature, which can result in coral bleaching. Heat-induced coral bleaching is primary a result of the expulsion of heat-stressed (and ROS-leaking) symbiotic dinoflagellate algae. The highly complex genomes of dinoflagellates presents problems with the development of reliable genetic tools. This means that our understanding of molecular mechanisms in dinoflagellates, including the heat-stress response, is poorly understood. We aim to expand the limited toolkit by developing transformation tools targeting endosymbiotic organelles with their relatively manageable genome sizes. Our research aims to establish dinoflagellate strains expressing ROS-sensitive fluorescence biosensors encoded in the chloroplast and mitochondria genomes of free-living and symbiotic dinoflagellate algae. This will allow us to monitor real-time ROS surges in response to heat stress.

A157

Mutual enrichment of *Streptomycetaceae* in the endosphere of barley and wheat suggests a key role for cereal crop health

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Abstract

Root microbiota provide plants with many benefits including increased nutrient bioavailability and protection from disease and abiotic stresses. A subsection of soil microbes are present in the plant root (endosphere) where microbial abundance is less diverse. A previous community profiling study demonstrated that the plant beneficial taxon *Streptomycetaceae* was highly enriched in the wheat root endosphere compared to the bulk soil (Prudence et al., 2021). We wanted to investigate this to see if the same relationship could be observed for barley, a related cereal.

Three different barley varieties were grown in the same field environment and the different root compartments (bulk soil, rhizosphere, endosphere) were separated and DNA subsequently extracted. Through sequencing of the 16S rRNA gene of the root compartments, we show that *Streptomycetaceae* are also highly enriched in the barley endosphere. We also demonstrate that other bacterial family abundances differ when comparing wheat and barley.

These results suggest that *Streptomycetaceae* play an important role for plants and leads us to speculate how *Streptomyces* is an effective coloniser of the root endosphere. We hypothesise that there are influencing factors that select for root endophytes either through soil composition, microbial competition or selection by the plant through secondary metabolites. Barley could prove to be a better model than wheat, due to a simpler, diploid genome. Access to knockout mutants could help us to pinpoint factors that influence *Streptomyces* enrichment.

Prudence, S. et al., 2021. Soil, senescence and exudate utilisation: characterisation of the Paragon var. spring bread wheat root microbiome. Environmental Microbiome.

A158

Construction of a synthetic microbial community of *Synechococcus elongatus* cscB/SPS and *Azotobacter vinelandii* ΔnifL using proteomics approach

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Abstract

Microbial communities play essential roles in various ecosystems and are exploited within different industrial sectors. Recently, attempts have been made to cultivate synthetic or artificial microbial consortia composed of defined species; however, details regarding their interactions and stability are often inadequate. A better understanding of functional changes would enable rational process monitoring, improving industrial productivity and control.

In this study, we established a synthetic microbial consortium based on the cyanobacterium *S. elongatus* cscB/SPS and nitrogen-fixing bacterium *A. vinelandii* ΔnifL, in which members can survive without carbon and nitrogen sources within their media. We hypothesise that this foundation can support a third member specialised in producing desired biomolecules, such as amino acid. Moreover, an approach for performing proteomics experiments and data analysis in a co-culture system has been established to investigate interactions among defined microbial consortia. Long term, the quantitative proteomics data will be used to modify process parameters to enhance symbiosis.

A159

Genome sequencing and prophage characterisation of Wolbachia strain wStr1

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Abstract

Wolbachia is a ubiquitous bacterial endosymbiont, estimated to be present in more than half of all insect species. In some of these associations it has profound effects on its hosts, commonly a range of reproductive manipulations which enhance its vertical transmission. The ability to edit the *Wolbachia* genome could be broadly applied to agricultural pest or disease control. However, as an obligate symbiont protected by a host-derived vacuole, *Wolbachia* is refractory to conventional editing techniques.

Phage WO is a temperate Caudovirales bacteriophage associated with *Wolbachia*. Lysogenic and pseudolysogenic phage WO contain genes critical to *Wolbachia*'s reproductive manipulations. When activated, the lytic phase is capable of penetrating both bacterial and eukaryotic cell membranes to effect escape or infection. Phage WO therefore represents a possible vehicle for editing *Wolbachia*. We characterised prophage presence in the wStr1 strain of *Wolbachia*, with the objective of identifying active phage.

We cultured wStr1 *Wolbachia* in SF9 host cells and extracted DNA. Sequencing was performed using Oxford Nanopore MinION and Illumina. Polishing was performed using Minimap2 to align ONT against Illumina reads and Racon/Pilon to generate consensus sequences. The final assembly comprises a single 1.7 Mb contig, a significant improvement over published data for wStr1. Initial annotation was performed with Prokka. Prophage Hunter tool was used to indicate regions of interest, marking 61 prophages of varying integrity. Two high scoring regions of ~30kb were annotated in greater detail using EggNOG mapper. These may represent active phage WO and will be the focus of further investigation.

A160

Escherichia coli EC958 (ST131) can resist phage infection in artificial urine by two different mechanisms

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Abstract

Bacteriophages have been used to treat bacterial infections for over 100 years, but until recently phage therapy was only used in a few countries. Today, the popularity of phage therapy in the rest of the world is increasing due to the antimicrobial resistance problem. However, the mainstream use of phage therapy still poses major challenges to overcome. One of the most important is to prevent bacterial phage-resistance mechanisms causing the treatment to fail. This project aims to study the resistance mechanisms of *Escherichia coli* EC958 (ST131) to phage LUC4: the best phage in our collection for this strain. By exploring the effect of subsequent phage-infections on the escape variants, we found that at least two different resistance mechanisms are in play during an 18-hour interaction culture in artificial urine. The first one involves a change in the phage receptor caused by SNPs or indels. Nevertheless, these variants are outcompeted by a subpopulation using a different and transient mechanism that is activated only while the threat of phage infection is present. Using RNA-seq we are currently investigating the genes potentially involved in this resistance mechanism. Understanding the causes of phage-resistance of a clinical *E. coli* strain may lead to solutions that can make phage therapy effective and safe to use in the wider population.

A161

Key factors of host cell physiology that impact on phage-host interactions

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Abstract

Pathogenic species of Mycobacterium require very long incubation periods (typically 8-18 weeks) for conventional culture and therefore DNA-based methods of detection are normally used. However, the robust nature of these acid-fast organisms reduces the efficiency of standard chemical or physical DNA extraction methods and limits the sensitivity of molecular diagnostic tests. These limitations have led to the development of phage-based detection methods, such as Actiphage®, that uses mycobacteriophage D29 as a viable DNA lysis reagent. However, there is limited understanding of the dynamic interactions between phage and host and it is known that the phage will only productively infect actively growing cells. Research to date show that D29 has a broad tropism and capable of infecting the pathogens Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium sbsp. paratuberculosis and the non-pathogen Mycobacterium smegmatis. However, the nature of the conserved receptor present on these different cells is unknown. In this study we found that host cells in stationary and mid-log growth phases exhibited different phage sensitivities, with the former being relatively phage-insensitive. To investigate the nature of the receptor, mid-log phase host cells were treated with proteinase K to remove surface-exposed proteins and rifampicin to prevent de novo synthesis of new proteins during incubation with the phage. This treatment led to a marked reduction of phage attachment efficiency that was reversible when the rifampicin was removed, allowing synthesis of new proteins. Together these observations suggest that a conserved protein facilitates infection and its presence on the cell surface is influenced by growth conditions.

A162

Whole genome profiling of Streptococcus pneumoniae identifies nine genes essential for penicillin tolerance

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Abstract

Invasive respiratory infections caused by *Streptococcus pneumoniae* are a common and often life-threatening disease found across the world. Traditionally these infections are treated with antibiotics, such as penicillin, which have massively reduced mortality rates for these diseases. However, there are a growing number of resistant *S.pneumoniae* strains emerging across the globe which threaten patient outcomes. This means, if we are to continue to treat *S.pneumoniae* infections with penicillins, we need to identify new points of weakness in bacterial cell metabolism.

Decades of work have shown *S.pneumoniae* penicillin resistance is linked to dramatic changes in their PBP enzymes. These modified PBP enzymes have reduced penicillin binding properties, yet they can still perform their essential function even in the presence of the antibiotic. However, what is less clear is what genes are required to support *S.pneumoniae* antibiotic tolerance and what is the fitness cost placed on the cell carrying these divergent PBP enzymes. In this study, we used a whole-genome fitness assay (Tn-seq) which identified nine genes required for *S.pneumoniae* penicillin tolerance. Loss of function of any of these loci increases penicillin sensitivity both in lab- and clinical strains.

This talk will discuss how these nine genes were identified by transposon sequencing, their effect on penicillin sensitivity across clinically important strains, and the function these proteins have in the *S.pneumoniae* cell.

A163

Investigating the structure of TolA and its relationship with the proton motive force

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Abstract

The outer membrane (OM) of Gram-negative bacteria provides a barrier to the outside world, conferring intrinsic resistance to many antibiotics. It is a non-energised membrane, which means that any processes carried out at the OM must utilise energy sources either in the cytoplasm or across the inner membrane (IM). The Tol-Pal system uses the proton motive force (PMF) across the IM to carry out its role in OM stabilisation during cell division.

The lipoprotein Pal forms a tether, interacting with the peptidoglycan (PG) and OM (Parsons, Lin and Orban, 2006). Binding of TolB to Pal abolishes PG binding (Bonsor *et al.,* 2007) and allows for recruitment of the complex to the divisome, where Pal accumulation is essential for maintaining the distance between the OM and PG (Petiti *et al.*, 2019; Szczepaniak *et al*., 2020). The IM stator complex TolQ-TolR modulates the binding state of Pal through the effector protein TolA. Following the formation of a TolQ-TolR-TolA complex, PMF-dependent conformational changes in TolA (Germon *et al.,* 2001) allow for its extension to bind TolB and retraction to remove TolB from Pal. This study examines the structure of TolA using a range of structural and biophysical techniques to better understand it's role in the Tol-Pal system's mechanism. In addition, a photoactivatable cross-linking strategy is used to study the molecular detail of the interaction between TolA and TolQ within the inner membrane. Combined, this data provides insight into how the TolQ-TolR-TolA complex forms and how subsequent conformational changes in TolA generate force.

A164

Examining the role of P. aeruginosa aminopeptidase aaaA in a simulated chronic wound model

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Abstract

Pseudomonas aeruginosa is a leading cause of bacterial wound infections and is associated with a disproportionately high level of mortality in burn patients. Because of its wide arsenal of virulence factors and biofilm formation ability, infections with *P. aeruginosa* are often chronic and extremely difficult to treat. In this study, an *in vitro* collagen-based simulated wound model is used to examine the role of the virulence factor AaaA (PA0328) in chronic wound infections. AaaA, or the Arginine-specific aminopeptidase of *P. aeruginosa* A, is a surface-tethered autotransporter which cleaves N-terminal arginine from peptides. In the oxygen and nutrient-limited environments of chronic wounds, this free arginine could serve as a nutrient source for *P. aeruginosa* via alternative metabolic pathways, or act as a signalling molecule involved in regulating biofilm formation and dispersal. As AaaA is surface tethered and immunogenic, it also has interesting potential as an antimicrobial drug or vaccine target. This study aims to probe the role of AaaA in the wound context, using a combination of transcriptional reporters, Western blotting, RT-qPCR and RNA-Seq to quantify and localise *aaaA* expression, and identify co-regulated genes. We've shown that in the wound infection model, AaaA is present on the transcriptional (lux-reporters and RT-qPCR) and translational level (Western blot). There is also some evidence from live/dead staining and CFU counting to suggest that *aaaA* may confer a survival advantage, which would mirror results seen previously *in vivo*. Further work is also underway to precisely localise AaaA within the wound biofilm using super-resolution imaging techniques.

A165

Investigating the mechanisms of dietary xylan degradation by key members of the human gut microbiota

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Abstract

The human gut microbiota (HGM) plays an essential role in the degradation of complex dietary polysaccharides, inaccessible to mammalian enzymes. Of the dominant HGM phylum, *Bacteroidetes,* two genera, *Bacteroides* and *Prevotella,* encode a particularly high abundance and diversity of Glycoside Hydrolase (GH) enzymes within their genomes. GHs are a diverse group of enzymes which function in the cleavage of glycosidic bonds between carbohydrate residues.

Xylans are beta-1,4 linked xylan polymers that are key components of the plant cell wall and hence have relatively high abundance within the human diet. Xylans vary in complexity, and highly decorated xylans, such as those from cereal crops, are recalcitrant to degradation by most xylan-targetting families of GHs.

Here we present structural and functional studies into the mechanisms involved in the breakdown of complex cereal xylans by *Bacteroides* and *Prevotella* spp. We show that enzymes belonging to the GH98 family possess endo-xylanase activity at the cell surface, specifically against highly-decorated corn xylan, with requirement for a xylose, arabinose doubly substituted xylose backbone residue for activity. Homologs to this enzyme are found in plant-pathogens and soil-dwelling bacteria, indicated a conserved function in xylan utilisation in a range of ecological niches.

Improved understanding of the utilisation mechanism of these complex cereal-grain xylans by key members of the HGM has potential wide-ranging applications, from nutritional sciences to the development of biofuels.

A166

Biochemical and structural studies of a nuclease substrate and cognate immunity protein of the *Staphylococcus aureus* type VII secretion system

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Abstract

The type VII secretion system (T7SS) of the pathogen *Staphylococcus aureus* is implicated in interbacterial competition and virulence. We previously identified a toxin named EsaD that is used to attack other *S. aureus.* Self-intoxication is prevented by an immunity protein, EsaG, which binds to the toxic C-terminal nuclease domain of EsaD. Genes encoding variants of EsaG are also accumulated by other *S. aureus* strains to provide protection against variants of EsaD from other attacking strains. Here we have investigated the interaction between EsaG and EsaD by X-ray crystallography. We use this and other biochemical knowledge to look at how natural variation in EsaD and EsaG homologues from competing strains relates to the interface between these proteins and their binding.

A167

Differential activity of efflux transporters of the model eukaryote *Dictyostelium*

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Abstract

The amoeba *Dictyostelium discoideum* inhabits soils, tracking bacterial and yeast prey through chemotaxis, and has a complex life cycle encompassing unicellular and multicellular stages. It has become a useful eukaryotic, biomedical, and cell biology model organism for phenomena from cell-type differentiation to signalling to phagocytosis. These social amoebae retain many similar genes to those of the human genome, including for the evolutionarily ubiquitous multidrug and efflux (MATE) transporters. Despite large multigene MATE families in some organisms, man and *Dictoystelium* both encode a pair. Each human MATE operates in specific tissues including the kidney and liver, and can facilitate cancer drug resistance. Their transcription in the model amoeba suggested one is more highly expressed and differentially regulated, with upregulation following 'test drug' treatment with polyphenolic secondary metabolites. The second MATE was upregulated most, and appeared to be the principal transporter of the pair, when toxin efflux was required. Fluorescence reporter visualisation confirmed the predicted plasma-membrane location in the unicellular amoeba. Genetic and biochemical ablation of each did not affect viability but diminished phagocytosis of prey bacteria and reduced the internal concentration of the polyphenolic treatments. Imaging and LCMS also confirmed MATE activity was efflux, not import. The function of these transporters has not been considered previously in multicellular *D. discoideum,* the sentinel cells of which were reported to sequester and remove toxins to the extracellular matrix which is left behind the moving 'slug'. It is also of interest when testing drug activity in this model organism.

A168

In silico analysis of the via locus and wider SPI-7 region in Vi-positive Salmonella Dublin strains

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Abstract

Salmonella is a pathogen of global importance, which elicits diverse clinical presentations depending on host and bacterial factors. Analysis of *Salmonella* genomes partially links systemic virulence with gain of function e.g., the virulence of *S.* Typhi is associated with clusters of genes, including the *via* locus, which is absent from *S.* Typhimurium. The *via* locus comprises 10 genes located on a 134kb region termed *Salmonella* pathogenicity island 7 (SPI-7). The *via* locus encodes the Vi antigen, a capsular polysaccharide that is a crucial virulence factor for *S.* Typhi. Studying the role of Vi *in vivo* has been challenging as *S.* Typhi is human-specific, however the *via* locus also occurs in some isolates of *S.* Dublin from cattle.

In silico analysis was conducted using Artemis and ACT to compare the *via* locus and wider SPI-7 regions of three sequenced Vi+ *S.* Dublin strains supplied by the Sanger Institute. They were also compared to a detailed annotation of *S.* Typhi CT18 and *S.* Typhi Ty2. The data shows that the entire *via* locus is highly conserved, however the SPI-7 region exhibits rearrangements in each Vi+ *S.* Dublin strain.

S. Dublin naturally causes typhoid-like disease within cattle, however the presence of Vi may enhance pathogenicity. The role of Vi *in vivo* has historically been researched using *S.* Typhimurium in murine models, which may not fully reflect events in natural hosts. Ongoing studies will probe the role of *S.* Dublin *via* genes in interactions with the bovine host, including by using novel surgical models to study invasion, inflammation, and systemic translocation.

A169

Understanding Carbohydrate active enzymes for degradation of bacterial plant pathogen biofilms

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Abstract

Many plant pathogenic bacteria produce biofilms to adhere to the hosts, induce vascular wilt and to persist in the environment. The biofilms produced consist of extracellular polysaccharides (EPS) which are varied and complex in structure. Studies have demonstrated the importance of EPS in the pathogenesis of the bacteria, bacteria genetically modified to no longer produce EPS have reduced or no pathogenicity. Additionally, EPS are vital for biofilms to offer protection from environmental stresses and disinfectants. Carbohydrate active enzymes (CAZymes) are proteins which are capable of degrading complex carbohydrates, including those from microbial sources. This project aims to identify and investigate the ability of specific CAZymes to degrade plant pathogenic EPS and so decrease the ability of the pathogen to cause disease and persist in the environment. EPS have been purified from two important plant pathogens *Erwinia amylovora* and *Xanthomonas campestris.* The structure of the EPS were characterised through monosaccharide analysis and NMR analysis. Crystal violet assays revealed the ability of specific CAZymes to disrupt and degrade biofilms formed by both pathogens. Further studies have shown the impact of enzymatic biofilm degradation on the efficacy of antibiotics and biocides against the pathogenic bacteria.

A170

Autoinducing peptide biosynthesis and quorum sensing inhibition in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus virulence is largely controlled by the *agr* quorum sensing system which responds to a pheromone autoinducing peptide (AIP). AIPs are produced by post-translational processing of precursor peptide AgrD by cytoplasmic endopeptidase AgrB. AgrD is directed to the cell membrane via an N-terminal amphipathic leader peptide. AgrB recognises AgrD and is predicted to form an enzyme bound intermediate resulting in cleavage of the AgrD C-terminus and formation of a thiolactone. Removal of the N-terminus releases the mature AIP. Once released externally, AIP binds to the histidine kinase AgrC on neighbouring cells activating a signal transduction pathway via AgrA, upregulating further AIP biosynthesis and virulence gene expression. The exact mechanism of AgrD processing is poorly understood.

Here an *agrBD* expression construct in *E. coli* was used to increase our understanding of AIP maturation. On detection of AgrB via western blot after co-expression of AgrBD, a shift in the size of AgrB was observed. Detection of the N-terminus of AgrD by western blot confirmed it was present in an AgrBD complex representing evidence for the enzyme bound intermediate of AgrD. This complex was not generated in the presence of AgrB protease inhibitors. This is being investigated further to identify the residues necessary for AgrBD complex formation. It will also be probed with AIP biosynthesis inhibitors such as ambuic acid to explore their mechanism of action. Investigation into AgrBD interactions should increase our understanding of AIP biosynthesis and aid development of inhibitors that target AIP biosynthesis for use as alternative therapies to prevent *S. aureus* infections.

A171

A Genome-Scale Antibiotic Screen in *Serratia marcescens* Identifies YdgH as a Conserved Modifier of Cephalosporin and Detergent Susceptibility

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Abstract

Serratia marcescens is adept at colonizing health care environments and is an important cause of invasive infections in vulnerable patients. Antibiotic resistance is a daunting problem in *S. marcescens* because, in addition to plasmid-mediated mechanisms, most isolates have considerable intrinsic resistance to multiple antibiotic classes. To discover endogenous modifiers of antibiotic susceptibility in *S. marcescens*, we subjected a high-density transposon insertion library to antibiotic selection. Subsequent analysis identified hundreds of candidate modifiers of susceptibility. Using single-gene deletions, we validated several candidate modifiers of cephalosporin susceptibility and chose *ydgH*, a gene of unknown function, for further characterization. Deletion of y*dgH* in *S. marcescens* resulted in decreased susceptibility to multiple cephalosporins and, in contrast, resulted in increased susceptibility to both cationic and anionic detergents. YdgH is highly conserved throughout the Enterobacterales, and we observed similar phenotypes in *Escherichia coli* O157:H7 and *Enterobacter cloacae* mutants. In follow-up experiments we have revealed YdgH to be a periplasmic monomer with a probable role in envelope homeostasis. Here, we present further efforts at elucidating the molecular mechanism of this conserved uncharacterized protein.

A173

Daptomycin-induced autolysis in *Bacillus subtilis*: *in vivo* evidence against membrane pore-formation as its principal mode of action

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Abstract

Daptomycin is a lipopeptide antibiotic that displays excellent activity against Gram-positive organisms and is often implemented as a last resort agent to combat multi-drug resistant bacterial infections. Despite this important clinical role, the precise mechanism of action of daptomycin is still heavily debated. In particular, much controversy is centred on whether or not daptomycin permeabilises the cytoplasmic membrane of target cells through pore-formation. Using a series of fluorescent microscopy-based techniques on the Gram-positive model organism *Bacillus subtilis*, we instead found that daptomycin triggers extensive autolysis; which has likely been misinterpreted as membrane pore-formation in previous *in vivo* studies. In cells lacking key cell wall-lytic enzymes, even prolonged incubation with daptomycin is incapable of inducing membrane pore-formation. In contrast, daptomycin causes a partial and heterogeneous depolarisation of the cytoplasmic membrane which, in turn, is responsible for inducing cellular autolysis. Importantly, a strong correlation exists between minimal growth-inhibitory concentration, induced partial membrane depolarisation, and the level of membrane potential required for supporting growth. Finally, we have demonstrated that daptomycin's recently identified interaction with the undecaprenyl-coupled cell wall precursor lipid II is not required for its membrane depolarising activity. Thus, daptomycin exhibits a dual mode of action: (i) lipid II-independent membrane depolarisation and autolysis and (ii) lipid II-dependent cell wall synthesis inhibition.

A174

Typing panel for phase variable elements of *Neisseria gonorrhoeae* lipooligosaccharide

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Abstract

Neisseria gonorrhoeae is a Gram negative bacterium, and a causative agent of sexually transmitted disease gonorrhoea. Lipo-oligosaccharide (LOS) is an essential component of the outer membrane and a potential target for novel therapeutics and vaccines which are needed to counter the rising antimicrobial resistance of the gonococcus. Furthermore, *N. gonorrhoeae* LOS is critical during interactions with the human host. However, research on gonococcal LOS is challenging because it is highly variable due to phase variation of enzymes involved its synthesis. Here we devised a novel scheme to characterise LOS expressed by *N. gonorrhoeae* strains employing a panel of available antibodies and lectins. Using a selection of wild type and isogenic knockout strains we show that the panel of reagents is specific for particular LOS structures. In addition, single cell resolution afforded by flow cytometry revealed that individual bacteria can express multiple LOS structures on their surface. Furthermore, we defined genotype:phenotype associations by sequencing phase variable *lgt* genes and assessing LOS structures in populations of bacteria. Finally, we applied sequencing and our LOS typing scheme to a range of current clinical isolates. Our findings provide a novel tool for gonococcal research, which should be informative about the potential coverage of vaccine and therapeutic approaches based on gonococcal LOS, and correlations between LOS structures and disease.

A175

Genetic diversity of the *Listeria monocytogenes* type VII secretion system and its secreted toxins suggests a key role in interbacterial competition

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Abstract

The type VII secretion system (T7SS) of Firmicute genera such as *Staphylococcus aureus* is implicated in interbacterial competition and virulence. A conserved membrane-bound ATPase protein, termed EssC, is a critical component of the T7SS and is the primary receptor for substrate proteins. We have previously identified seven distinct EssC variants across *Listeria monocytogenes* that differ in their C-terminal region; each variant co-occurs with distinct subsets of genes encoding secretion substrates and accessory proteins. Across these *L. monocytogenes* strains we also identified 40 different T7SS substrates containing an N-terminal LXG domain, most of which are encoded at conserved genomic loci. We now report a newly identified eighth *essC* variant, predominantly restricted to a subset of Lineage III isolates of *L. monocytogenes*. Downstream of *essC8* is an extremely large gene encoding a predicted toxin comprised of multiple RHS-repeat domains. RHS proteins have previously been identified as substrates of the Gram-negative type VI secretion system, but this is the first time an RHS substrate has been genetically linked to a T7SS. Ten further novel LXG-domain toxins have been identified that are unique to *essC8* strains, encoded at new and pre-established loci. Lineage III strains of *L. monocytogenes* are predominantly isolated from ruminant species, and we hypothesise that these novel T7SS toxins may provide a competitive growth advantage against other bacterial species in the gastrointestinal environment.

A176

Lipid A modification and polymyxin resistance in an emerging CF-pathogen

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Abstract

Achromobacter species are Gram-negative bacterial opportunistic pathogens that infect people with cystic fibrosis (PWCF) and also non-CF individuals. Chronic pulmonary infection in PWCF is often treated by nebulisation with polymyxin/colistin. Polymyxins interact with the negatively charged lipid A moiety of the lipopolysaccharide (LPS). Disruption of this interaction by the addition of a positively-charged molecule to the phosphate of lipid A can confer resistance to polymyxins and affects LPS interactions with innate immune receptors. The pathogenesis of *Achromobacter xylosoxidans* infection is not well understood and no studies are reported on the LPS of the members of this genus. Using MALDI-TOF mass spectrometry, we identified modifications in the lipid A structure of *Achromobacter* isolates after challenge with polymyxin B (PmB), which include the gain of a glucosamine modification, loss of phosphate groups, and acyl chain alterations. The putative genes responsible for these modifications were deleted in an *Achromobacter insuavis* clinical strain. Deletion of *pagP* and *pagL* homologues, responsible for changes in acylation, resulted in small reductions in PmB resistance. In contrast, the deletion of *arnT* - the gene responsible for glucosamine modification - led to a dramatic loss of PmB resistance (>258µg/mL to <1µg/mL), virulence attenuation in the *Galleria mellonella* infection model, and bacterial defects in septation. We conclude that the ArnT modification of LPS is critical not only for PmB resistance but also for the physiology of the bacterium in the context of cell division and biogenesis of the cell envelope.

A177

Characterisation of a Toxin Encoded by the Type VII Secretion System of *Staphylococcus aureus* Strain EMRSA-15

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Abstract

The secretion of protein effectors is an essential process that allows bacteria to readily adapt to changing and challenging environments. A number of virulence factors are known to be exported by complex secretion systems that can modulate host immune responses. One such system is the Type VII secretion system (T7SS), components of which have been discovered in the human pathogen *Staphylococcus aureus*. It is now becoming clear that the T7SS has a role in interbacterial competition, as substrates belonging to the LXG and YeeF protein families have been shown to demonstrate antibacterial activity.

Analysis of *S. aureus* genomes has led to the discovery that this bacterium encodes four types of T7SS. While genes encoding the core machinery are conserved between each type, variability in the T7 operon begins at the C-terminus of *essC*, a gene that encodes an ATPase essential for secretion. Downstream from *essC*, and unique to each variant, are genes predicted to encode toxins, immunities and chaperones. Current work has shown that the T7 operon of *S. aureus* strain EMRSA-15 encodes a protein with toxic activity. This toxin is lethal to both *S. aureus* and *Escherichia coli*, indicating that its target is common to both Gram-negative and Gram-positive bacteria. Using gene deletion studies, bacterial two hybrid assays and co-immunoprecipitations, the immunity protein required to neutralise toxic activity has been discovered, as well as a candidate chaperone. Over-production of the toxin has been shown to result in membrane depolarisation and the mechanism behind this is currently being investigated.

A178

Metal-dependent Surface Layer Biogenesis in *Caulobacter crescentus*

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Abstract

Surface layers (S-layers) are proteinaceous crystalline coats that constitute the outermost component of most prokaryotic cell envelopes. S-layer proteins are a diverse class of molecules able to self-assemble into regular two-dimensional lattices, fulfilling a variety of biological functions, often in a metal dependent manner. We have investigated the roles of metal ions in the formation of the *Caulobacter crescentus* S-layer, using high-resolution structural and cell biology techniques combined with molecular simulations. Our optical microscopy of fluorescently tagged S-layers demonstrates that calcium ions make an essential contribution to the formation of the S-layer on cells by facilitating both lattice formation as well as binding to the cell surface. We also report all-atom molecular dynamics simulations of the complete S-layer lattice, which reveal the importance of multiple metal ions bound to the S-layer. Finally, using electron cryomicroscopy structure determination along with long-wavelength X-ray anomalous diffraction experiments, we have confirmed the identities and mapped the positions of metal ions in the S-layer at near-atomic resolution, confirming all our predictions from the cellular and simulations data. These findings contribute to our understanding of how *C. crescentus* cells are able to form a regularly arranged S-layer on their surface, with implications on both fundamental prokaryotic biology related to S-layers and the synthetic biology of biomaterials.

A179

Functional and structural analysis of the ESX-1 dependent substrate EspE in *Mycobacterium marinum.*

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Abstract

Mycobacteria have acquired specialized Type VII secretion systems in order to secrete substrates across their uniquely hydrophobic cell envelope. A T7SS subtype, called ESX-1, is one of the most important virulence factors of *Mycobacterium tuberculosis* and *Mycobacterium marinum*, because of its essential role in phagosomal rupture and subsequent mycobacterial translocation to the cytosol of infected macrophages. The ESX-1 dependent substrate EspE is abundantly present on the cell surface of the model organism *Mycobacterium marinum.* Here, an EspE frameshift mutant was made using the genome editing tool CRISPR1-Cas9. Analysis of the *EspE fs* mutant showed a defect in hemolytic capabilities and zebrafish infection compared to the wild-type. Episomal complementation of the *EspE fs* mutant with the *espE/espF* operon containing a C-terminal Strep-tag on the *espE* gene did not interfere with its secretion. Affinity purification from the cytosolic compartment of an ESX-1 mutant showed stable complex formation of EspE.strep before secretion over the inner membrane. This complex was ~145kDa and contained multiple proteins. Extraction of EspE.strep from the cell surface of the complemented *EspE fs* mutant, using the mild detergent Genapol X-080, showed larger EspE complexes of ~500kDa. Removing the detergent from the purification sample resulted in increased complex sizes of EspE up till 1.2mDa. Electron microscopy revealed a globular particle for the smallest EspE complexes that filaments to form larger complexes. In summary, EspE plays an essential role within ESX-1 dependent virulence by forming large filamentous complexes on the cell surface which were analyzed in its native environment.

A180

Building and Loading the Type VI Secretion System Sheath from *Pseudomonas aeruginosa*.

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Abstract

Pseudomonas aeruginosa builds-up a double-membrane spanning Type VI secretion system (T6SS) to inject toxic effector proteins to bacterial, fungal or mammalian cells. The *P. aeruginosa* T6SS is shaped by a membrane complex, a baseplate, and a contractile tail made by a sheath and a Hcp-VgrG device on which the effectors are loaded. Importantly, during T6SS assembly, baseplate proteins are recruited onto the membrane complex by the TssA protein. TssA proteins also orchestrate sheath extension and assist sheath stabilization. TssA proteins can be grouped in two main classes, long (*ca* 500 amino acids) and short (*ca* 350 aa) and achieve their functions by different mechanisms. We present the structural characterisation of different TssA proteins from *P. aeruginosa* and unveil a plausible mechanism for T6SS sheath assembly and cargo effectors loading in the Hcp tube prior injection into target cells.

A181

Understanding the Binding Interactions of *Staphylococcus aureus* Fibronectin Binding Protein B

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Abstract

In atopic dermatitis, *Staphylococcus aureus* has increased adherence to the skin due to changes in the stratum corneum which include increased display of the glycoprotein corneodesmosin (CDSN). It has previously been shown that *S. aureus* adheres to CDSN within the region of the N-terminal glycine-serine rich loop and this is mediated via clumping factor B (ClfB) and fibronectin binding protein B (FnBPB) in the *S. aureus* atopic dermatitis strain AD08. The interaction of ClfB and other related proteins with fibrinogen, another *S. aureus* ligand, has been demonstrated to be via a ligand binding trench within the N2N3 subdomain. We are utilising a selection of modelling, mutagenesis, structural biology and biochemistry to further confirm if the binding of FnBPB to CDSN is analogous to that of fibrinogen. At present we have co-purified a complex of the CDSN N-terminal loop and FnBPB N2N3 subdomain using affinity tagged purifications, which we currently have in crystal trials. Following modelling predictions we carried out mutagenesis within the ligand binding trench of the recombinant proteins. Our results demonstrate that mutations at N312A and F314A impact on binding to fibrinogen and CDSN, indicating a role of the ligand trench of FnBPB in the binding to both of these ligands. There are further ongoing investigations to define additional contacts between FnBPB and its ligands, as well as comparative studies exploiting the natural variation that exists between the FnBPB proteins produced by *S. aureus* strains from different clonal complexes.

A182

Recombination drives the evolution of immunity gene repertoires of the Type VII Secretion System in Staphylococcus aureus

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Abstract

The Type VII Secretion System (T7SS) is a protein secretion system found in Actinobacteria and Firmicutes. Two substrates of the T7SS have been characterised in *Staphylococcus aureus*, both of which are polymorphic toxins that target bacteria. EsaD is a nuclease toxin that is neutralised by the cognate immunity protein, EsaG, while TspA is a membrane-depolarising toxin, neutralised by the TsaI immunity protein. The *S. aureus* RN6390 strain encodes 12 tandem, non-identical copies of *esaG* and 11 tandem, non-identical copies of *tsaI*. To understand how immunity gene repertoires evolve, I undertook sequence analysis of *esaG* and *tsaI* genes.

An alignment of *esaG* homologues identified three regions of high homology within the genes and their downstream intergenic regions. RDP4 software was used to predict recombination events between these genes in the *S. aureus* NCTC8325 lineage. My analysis showed that many recombination events have occurred between these genes, mediated through the regions of high homology. These events lead to loss or expansion of *esaG* numbers, as well as shuffling between sequences. I observed similar recombination events in an epidemic strain of *S. aureus* USA300, leading to the deletion of several *esaG* copies.

An alignment of *tsaI* homologues identified a large region of homology at the beginning of these genes. Whilst recombination events were not predicted between the genes, it is likely that gene duplication events occurred to increase the size of this gene repertoire. My analysis has revealed a mechanism for *S. aureus* strains to rapidly evolve immunity genes in response to new toxin variants.

A183

The bacterial Sec-machinery: a target for new strategies against antimicrobial resistance

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Abstract

Despite the rising threat of antimicrobial resistance, recent decades have seen a decline in antimicrobial discovery. Target-based antimicrobial drug discovery describes the identification of appropriate targets and establishment of bespoke assays for inhibitors of those subjects. The bacterial Sec-machinery, a major system of protein transport across and into the cytoplasmic membrane, is essential for all bacteria, differs from its human counterpart and contributes to the virulence and antimicrobial resistance of many pathogens; namely, the export of β-lactamases. It therefore represents a promising target for development of antimicrobial drugs, both as a stand-alone target and in terms of re-potentiating β-lactam antibiotics. We have established a whole-cell split-luciferase-based assay to monitor inhibition of the Sec-machinery. After validation with a model Sec substrate and known inhibitors of the machinery, the assay was scaled up for use in a local screen of 5000 diverse synthetic compounds, giving an average Z'-factor of 0.71 and confirmed hit rate of 0.12%. Secondary assays demonstrated that the strongest hit has specific inhibitory activity at concentrations ranging from 10 to 200 µM. The whole-cell assay has also been adapted for analysing export of metallo-β-lactamases IMP-1 and NDM-1, yielding data consistent with traditional assays for β-lactamase activity.

A184

Inhibition of autophagy during early stages of Semliki Forest virus infection

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Abstract

Autophagy is an autonomous process in which cytoplasmic cargo and cellular debris are delivered to lysosomes for degradation in specialised double membrane vesicles called autophagosomes. Recent research has focussed on upregulation of autophagy during growth of RNA viruses, and autophagy inhibition as potential treatment for virus infection. Semliki Forest virus (SFV) is a positive-sense single stranded RNA virus from the alphavirus genus. Previous research has revealed that accumulation of autophagosomes during late stages of SFV infection resulting in increased virus production. Chikungunya, another alphavirus showed the same autophagosome accumulation during early stages of infection and an increase in virus titre. Infection of mice with Sindbis virus expressing Beclin-1 supressed replication in the brain reducing mortality. Additionally, depletion or disruption of ATG5 in mice infected with Sindbis resulted in increased mortality. Here, autophagy was inhibited in neuroblastoma cells prior to SFV infection using 3-Methyladenine and Spautin-1. Fluorescence microscopy was used to analyse LC3B, and VPS34 puncta and RT-qPCR to monitor viral genome levels in early stages of infection. Flow cytometry was used to quantify levels of LC3 expression and SFV nsP1 and plaque assays to measure infectious virus production. Results showed inhibition of autophagy reduced SFV replication during early stages of infection and this may be related to virus entry and autophagosome accumulation. Further studies will use electron microscopy and small molecule fluorescence to track virus movement in membranes and protein-protein interactions. This research provides evidence that autophagy has the potential to be exploited as a treatment for neurovirulent viruses.

A185

Investigating the interaction between Influenza viral protein M1 and host factor Transportins during virus uncoating

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Abstract

Influenza viruses cause a significant disease, medical and economic burden, with Influenza A viruses (IAV) causing periodic epidemic and pandemic influenza disease among the human population. Further understanding of the host-viral protein interaction during virus uncoating can help identify potential novel therapeutic strategies. A recent study identified Transportin-1 (TNPO1), a protein involved in nuclear import of RNA-binding proteins to be exploited by IAV during the virus uncoating stage of the life cycle to enable efficient Matrix Protein 1 (M1) uncoating of viral ribonucleoproteins (vRNPs) and their subsequent nuclear import. In this project, key residues which are potentially important in this interaction were substituted through site-directed mutagenesis of the M gene of Influenza A and B viruses. Mutant M1 viruses containing single and double substitutions in the M1 protein were generated using reverse genetics through the eight bi-directional plasmid system and passaged twice in MDCK cells to check for the stability of the substitution. The growth kinetics of the mutant M1 viruses were tested and compared to the wild-type virus. Furthermore, using indirect immunofluorescence assay techniques in combination with siRNA-transfected cells targeting TNPO1, 2 and 3, various influenza virus subtypes including the mutant M1 viruses were tested for their transportin dependency and impeded replication to help inform which residues on the M1 protein are most critical.

A186

A toolbox for investigating RNA-RNA interactions in viruses

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Abstract

Functional intra-molecular long-range RNA-RNA interactions (LRIs) play a multitude of essential roles in regulating virus replication. Similarly, growing experimental evidence suggests that flexible inter-molecular LRIs between viral genomic RNAs are pivotal for selective assembly and packaging of segmented RNA genomes in members of the *Orthomyxoviridae* and *Reoviridae* families. Although tools exist for predicting conserved LRIs in RNA viruses, such tools rely on the sequence conservation analyses, thus lacking the capacity to detect flexible, often strain-specific LRIs, similar to those recently described in influenza A virus (IAV) strains. Here, we present a new toolkit for detecting and analysing flexible and strain-specific intra- and inter-molecular LRIs in viral RNA genomes. Our tools predict both types of LRIs independently, and allow for further experimental validation by incorporating experimental data from various structure probing and RNA cross-linking tools, including SHAPE-MaP, SPLASH, 2CIMPL etc. Using publicly available SPLASH data for various IAV strains, we show that our tool reliably detects, on average, over 91% of the inter-segmental LRIs identified by SPLASH. Furthermore, our toolbox can be used for designing synonymous and compensatory mutations for validation of functional LRIs in reverse genetics experiments.

A187

Perceptions of Peers' Open Science Attitudes in the Influenza Community Lead to Less Open Science Behaviours by Influenza Researchers, Despite Personal Support for Open Science

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Abstract

A lack of transparency is not only a crisis in the social and behavioural sciences -- it is also an issue in microbiology. While practices such as pre-registration and data sharing have spread in some communities, they are not widespread in areas like pre-clinical influenza research. This has become evident as a result of the COVID-19 pandemic, which has catalysed the rapid adoption of pre-printing. Prior to the pandemic, scientists reported that pressure to publish and selective reporting often contributed to issues with reproducibility. To this point, replications and null results reporting are often overlooked for publication because cultural incentives emphasize novelty over verification, indicating a disconnect between the push for better science and the academic publishing community. To learn more about open science in the microbiology community, the Open Scholarship Survey asked influenza researchers (N = 228) about their beliefs, behaviours, and perceptions regarding pre-registration and null results reporting. Overall, the influenza community reported more favourable attitudes toward pre-registration (43%) and null results reporting (78%) than they perceived their peers to have (39%, 28%). This disconnect may partially explain why influenza researchers do not engage in these practices regularly. A minimal number of respondents indicated that they included a pre-registration (20%) or null result (15%) in their most recent publication. We hope that illuminating the overall favourability of researchers toward open science practices will support policy initiatives that will lead to a more transparent influenza research process, and hence to more robust research on this major human pathogen.

A188

Stabilization of Focal Adhesions by HSV-1 pUL7:pUL51

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Abstract

Herpes simplex virus (HSV)-1 tegument proteins pUL7 and pUL51 form a stable complex and promote efficient virus assembly and cell-to-cell spread. Our previous studies have shown that the complex co-localises with *trans*-Golgi membranes and with focal adhesions, both during infection and when transfected together into cultured cells. Cells infected with HSV-1 lacking pUL7:pUL51 detach more readily from their substrate, suggesting that this complex stabilizes focal adhesions (and thus cell attachment) during infection. We now seek to understand the mechanism by which pUL7:pUL51 is recruited to focal adhesions and how such binding affects virus cell-to-cell spread.

Expressing pUL7:pUL51 outside the context of HSV-1 infection modifies cell motility, suggesting that it directly modulates focal adhesion turnover. Using biotin proximity ligation (BioID) and quantitative mass spectrometry we identified an integrin-binding cellular multi-protein complex as the putative pUL7:pUL51 binding partner at focal adhesions. Immunofluorescence microscopy shows extensive co-localization of pUL7:pUL51 and this focal adhesion protein complex outside the context of HSV-1 infection. Additionally, this complex efficiently co-immunoprecipitates with pUL7:pUL51 expressed outside the context of infection, suggesting that the interaction does not require other viral proteins. Further molecular characterization of this interaction using purified components is ongoing, with the aim of obtaining high-resolution structural information to facilitate site-directed mutagenesis and dissect the contribution of focal adhesion binding to virus replication and cell-to-cell spread. Understanding how HSV-1 modulates focal adhesions could accelerate the search for an effective HSV-1 vaccine and illuminate new strategies for inhibiting cancer metastasis and other integrin adhesion complex (IAC) associated pathologies.

A189

Molecular characterisation of the Bacillus subtilis SPβ bacteriophage viral particle

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Abstract

Bacteriophages are key elements for the intricate ecology of their bacteria hosts. One of the most striking features of these viruses is that they are found in nature with an enormous array in their bacterial hosts and lifestyles, their genetic content, or the components of the viral particle. The bacteriophages of the SPBeta family infecting *Bacillus subtilis* are temperate phages that belong to the Siphoviridae family of double-stranded DNA viruses. The model for this family, the phage SPβ was firstly identified in the 168 strain of *Bacillus subtilis*, and with 138,418 bp and 188 ORF it has been recently characterise as encoding a quorum sensing system that allows the phage to sense the surrounding population to control their life cycle. In this work we have further characterise this enigmatic phage, by performing a systematic search for the proteins involved in the production of the viral particle. We have identified 7 of these proteins that were present in the viral particle, and we have further identified 5 additional proteins needed for the packaging of the phage. In this work we have characterise the packaging module of the SPβ bacteriophage and identified, for the first-time new proteins involved in the packaging process of these family of phages.

A190

Effect of antiandrogens on the tropism of seasonal human coronaviruses

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Abstract

SARS-CoV-2, the causative agent of COVID-19 causes mild to severe lower respiratory infection. The common human coronaviruses (HCoVs: OC43, 229E, HKU1 and NL63) are endemic viruses that cause 10-30% of common colds and occasionally more severe disease in immunocompromised patients or the elderly. HCoVs share respiratory tropism with SARS-CoV-2 suggestive of a shared cell receptor (ACE2) by at least HCoV-NL63 and cleavage of the spike proteins by the protease Transmembrane Serine Protease 2 (TMPRSS2). Multiple studies have shown that TMPRSS2 is an androgen receptor (AR) target gene. AR exerts most of its effects in sex hormone-dependent body tissues but is also expressed in the lung. We and others demonstrated that antiandrogens reduce TMPRSS2 expression and prevent SARS-CoV-2 entry in vitro and in vivo. The TMPRSS2 cleavage site is well conserved in HCoVs and has been shown to be associated with 229E and NL63 cell entry. Herein, we hypothesized that modulation of AR signaling either during infection or in response to treatment with AR antagonists might determine the outcome of HCoV infection. Leveraging publicly available and our own transcriptome data, we found that infection of lung cells with 229E and SARS-CoV-2 induces a subset of AR-signaling dependent genes suggesting a cross-talk between CoV infection and AR signaling. To assess the effect of antiandrogens in HCoV infection, we generated pseudotyped HCoVs and screened a range of commercially used antiandrogens with a luciferase assay. The identification of the effect of (anti-)androgens in HCoV infection could potentially impact therapeutic responses in coronaviruses' diseases.

A191

Development of tools to study the mechanism of chronic liver disease associated with hepatitis C virus genotype 3 infection

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Abstract

Hepatitis C virus (HCV) infection is the leading cause of liver transplantation, cirrhosis and cancer. HCV genotype 3 (GT3), the second most common genotype worldwide, shows a high level of resistance to newly developed antivirals and a more rapid progression to chronic liver disease. To understand the mechanisms underpinning the pathology of GT3 infection we plan to exploit human precision cut liver slices (PCLS) which are viable for up to 6 days, presenting as a reliable *ex vivo* model for the investigation of liver pathology.

To facilitate these studies we are modifying a subgenomic replicon and infectious clone of GT3 (DBN3a) by inserting tags into domain III of NS5A. Unlike other HCV GTs which can tolerate the insertion of both short tags and fluorescent fusion proteins well, the generation and assessment of NS5A tagged HCV GT3 clones for replication suggested that genetic modification is best tolerated and limited to the most downstream point of the C terminus. Furthermore, this site can only tolerate a twin streptactin tag (TST) and not the insertion of GFP. This suggests HCV GT3 NS5A domain III may have different functions in comparison to other HCV GTs. Future research will prioritise optimising HCV infectious clones prior to infection of PCLS. PCLS will be utilised alongside proteomic studies to investigate GT3 and NS5A specific contributions to HCV pathology.

A192

Ancient herpes simplex virus 1 genomes reveal recent viral population structure in Eurasia

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Abstract

Human herpes simplex virus 1 (HSV-1) causes lifelong infection, infecting billions of adults globally. Simplexviruses are common among primates, leading to a hypothesis that HSV-1 co-diverged with anatomically modern humans migrating out of Africa. Phylogeographic clustering of sampled HSV-1 genomic diversity into European, pan-Eurasian, and African groups has been taken as evidence that viral genetic diversity is the result of ancient human migrations, although a much younger origin for these patterns has also been proposed.

Ancient DNA data can help to resolve this conundrum. Until now, the lack of ancient HSV-1 genomes, high rates of recombination, and high mobility of humans in the modern era have impeded our understanding of the evolutionary history of HSV-1. Here we present three full and one partial ancient European HSV-1 genomes, covering the period of the 3rd to the 17th century CE, sequenced to up to 9.5× coverage. These HSV-1 strains fall within modern Eurasian diversity. We estimate a mean mutation rate of 7.6×10^{-7} - 1.13×10^{-6} for non-African diversity leading to an estimated age of sampled modern Eurasian diversity of 4.7 kya (thousand years ago), more recent than estimates derived from co-divergence of humans and HSV-1. Extrapolation of these rates indicate the age of sampled HSV-1 to 5.3 kya, considerably more recent that the divergence of human populations. This suggests lineage replacement in the distribution of HSV-1 through Eurasia coinciding with Bronze Age migrations. Even in pathogens causing lifelong latent infections, new variants may replace old lineages.

A194

Novel *in vitro* and *ex vivo* models for investigating Hepatitis E Virus infection

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Abstract

Hepatitis E virus (HEV) is a leading cause of hepatitis worldwide, and the only zoonotic hepatitis virus. In developed countries across Europe HEV genotypes 3 and 4 are transmitted to humans through the consumption of pork products. The virus is widespread throughout commercial pig herds, with a recent U.K. study demonstrating HEV RNA in both faeces and organs in 20% of pigs at slaughter. In humans, infection with HEV often results in acute disease but can progress to chronic disease in immunocompromised individuals and those with pre-existing liver disease. In contrast, current evidence suggests that HEV infection of pigs is asymptomatic. The absence of efficient *in vitro* or *ex vivo* model systems for the investigation of this emerging zoonotic virus means that little is currently known about HEV biology and pathogenesis. Indeed, a recent report commissioned by the U.K. Food Standards Authority stated that the lack of a robust cell culture model for the study of HEV infection is the greatest roadblock to our understanding of factors that determine HEV transmission and to the development of novel antiviral treatments for HEV. We have optimised novel *in vitro* and *ex vivo* human and porcine model systems and employed them in investigating the early stages of HEV infection and replication. Critically, these models support infection and replication of HEV genotype 3, human clinical isolates and provide an invaluable tool that will facilitate the investigation of this important emerging zoonotic pathogen.

A195

Does The Complement System Influence Ebola Virus And SARS-CoV-2 Pathogenesis?

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Abstract

The complement system is a component of innate immunity that is capable of responding to viral infections and mediating a variety of immune functions including inflammation, chemotaxis, and neutralisation. Activation of complement system has previously been implicated in *Ebolavirus* and *Coronavirus* infections, yet the molecular components underlying its role in enhancing or abrogating their pathogenesis is largely under-researched. Using ELISAs we identified novel interactions of *Ebolavirus* and SARS-CoV-2 viral proteins with the lectin complement pathway and subsequent complement deposition. These interactions have the potential to influence pathogenesis *in vivo* by mediating inflammation, chemotaxis, and neutralisation. Using flow cytometry assays we characterised two cohorts of EBOV convalescent plasma based on their neutralisation and IgG titres, and assessed their capacity for antibody-dependent complement deposition (ADCD) against various *Ebolavirus* proteins. The ability to mediate ADCD was previously shown to be protective in *in vivo* mouse models for EBOV. We found differences in the ability to mediate ADCD depending on the IgG titre and/or the *Ebolavirus* protein used. Lastly, we assessed the functional effects of these interactions using wild-type neutralisation assays supplemented with complement. The complement system is typically excluded from immunoassays but it can affect antibody function. We showed for the first time that convalescent plasma together with complement can result in a significant enhancement to antibody-mediated neutralisation of wild-type EBOV. Work is ongoing to assess whether the same effect is observed with antibody-mediated neutralisation of wild-type SARS-CoV-2.

A196

Dengue virus (DENV) non-structural protein-1 (NS1) and its serotypic effects on the liver's microvasculature

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Abstract

DENV is the most prevalent arbovirus in humans, with a small percentage attaining severe dengue, which comprises of potentially fatal cardiovascular complications, including vascular leakage, haemorrhage, organ failure and shock. Presently, the understanding of the mechanisms leading to microvascular leakage remain incomplete. While our group has showcased DENV-2 NS1 to disrupt endothelial-pericyte interactions and enhanced endothelial cell permeability *in vitro*, it is unclear whether this holds true across DENV's four serotypes. It is also uncertain whether DENV NS1 affects all types of pericytes throughout the body similarly. Our previous work utilised human umbilical vein endothelial cells (HUVECs) and saphenous venous pericytes (SVPs), which has now been extended to include hepatic stellate cells (HSCs), since the liver is a significant site of dengue pathology. Transendothelial electrical resistance readings of co-cultures of HUVECs with SVPs and HSCs both revealed the DENV serotypes to significantly drop endothelial membrane function, particularly at DENV-2. Additionally, the 3D Matrigel angiogenesis assay revealed the DENV serotypes to alter HUVECs and SVP's ability to form vascular structures. The MTT proliferation assay utilising HSCs showcased liver pericytes to increase proliferation after DENV exposure, with immunocytochemistry of HSCs also revealing a rise in myofibroblast protein alpha-smooth muscle actin. These results collectively show the disruption of endothelial-pericyte interactions is shared across the DENV serotypes and suggest this loss is due to liver pericyte differentiation. This information gives further insight to the mechanisms involved with DENV interactions with the vasculature, which could help establish a future diagnostic test or treatment.

A197

Differential viral pathogenesis of Crimean-Congo Hemorrhagic Fever Virus infected human and bovine cell cultures identified by microRNA array and in vitro experiments

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Abstract

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick related deadly zoonotic disease with severe clinical manifestation in human, but without symptomatic disease in wild or domestic animals. The factors responsible for the different outcomes are still unknown. To determine the contrasting viral pathogenic pathways, we compared infected bovine- and human secondary and primary kidney cells by in vitro and microarray assays.

Interestingly, in vitro and microarray experiments showed that antiviral immune responses, apoptotic pathways and platelet function related viral pathogenesis exhibits major differences in the expression of human and bovine host genes/miRNA pathways. MiRNA related gene regulation causes cell damage in human kidney cells through upregulation of cytokine-, oxidative stress- and apoptotic pathways. In contrast, we concluded major decrease in cell-damaging apoptotic and oxidative stress-related pathways and increase in protecting Treg pathways in bovine kidney cells. Furthermore, platelet production which is an important prognostic factor in CCHFV infected human patients, were found to be downregulated in human cells and upregulated in bovine cells. We propose, that similarly to other members of the Bunyaviridae family, CCHFV modulates Potassium channels during viral entry. Related genes were upregulated in human while downregulated in bovine cells.

This is the first microarray report of CCHFV susceptibility and replication patterns in bovine cells and the first report to compare human and a natural host viral susceptibility. Our results give potential answers for the differences in human and reservoir pathogenesis. Our candidate microRNAs may have broad and important implications for therapeutic approaches.

A198

Uncovering the interactome of novel influenza proteins using mass spectrometry

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Abstract

Viruses depend on their ability to interact with host proteins for their replication. We recently identified a class of cryptic proteins encoded by influenza viruses, but the interaction partners of these proteins are unknown. These proteins (called upstream proteins, UP) are synthesised thanks to the random presence of upstream start codons in the 5' end of host mRNAs which are cleaved and used for priming influenza mRNA synthesis. This work aims to study the interactome of two UPs that we had previously shown to contribute to virulence: NP-ext, an N-terminal extended version of the viral nucleoprotein (NP), and PB1-UFO, a novel frameshifted protein whose gene overlaps that of the viral PB1 protein. The protein structure prediction algorithm Alphafold suggested that both PB1-UFO and the novel sequence in NP-ext are unstructured, suggesting that they could have flexibility in forming protein-protein interactions. To study the capability of NP-ext and PB1-UFO for such interactions, we are using affinity purification combined with liquid chromatography and tandem mass spectrometry (AP-LC/MS-MS) to characterise their interactomes in both uninfected and infected cells. The UP interactomes will be compared to those of the canonical viral gene products (NP and PB1). Unique protein-protein interactions will then be validated and further characterised in order to uncover their role in infection. With this study, we will gather valuable insight into the role of the recently-discovered influenza upstream proteome.

A199

Bacterial compounds enhance bluetongue virus infection in blood derived purified bovine monocytes

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Abstract

Bluetongue virus (BTV) is the causative agent of bluetongue, an OIE notifiable disease of ruminants. BTV is transmitted during a blood meal of its primary insect vector, *Culicoides* biting midges. As observed for other arboviruses and their insect vectors, the saliva of *Culicoides* can enhance BTV infection. Specifically, it has been shown that a non-proteinaceous bacterial component of the saliva plays a key role in the enhancement of BTV infection in monocytes within bovine peripheral blood mononuclear cells (PBMCs).

In this study, the role of bacterial components, such as Lipopolysaccharides (LPS), was further assessed for their ability to enhance BTV infection in blood-derived purified bovine monocytes.

Bovine PBMCs were isolated and $CD14^+$ monocytes purified by positive selection. Subsequently, monocytes were infected with BTV-1 only or BTV-1 in the presence of E. *coli* or S. *enterica* LPS, or detoxified *E. coli* LPS. The expression of BTV viral structural proteins (VSPs) and non-structural protein 2 (NS2) was then analysed by flow cytometry and/or confocal microscopy. Both *S. enterica* and *E. coli* LPS significantly increased expression of VSPs and NS2 compared to BTV-1 only, demonstrating that LPS enhances BTV infection in bovine monocytes. Interestingly detoxified *E. coli* LPS did not seem to have the same effect. Further work is required to investigate not only the infection dynamics but also the mechanism of enhancement of LPS and bacteria on BTV infection.

Overall, our study suggests that BTV might exploit bacterial compounds and/or bacteria to enhance infection which further highlights the complexity of arbovirus-vector-host interactions.

A201

Responses of pediatric airway epithelium to viral and/or aeroallergens in health and disease

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Abstract

Asthma affects 300 million children worldwide. Early age viral infection and allergen sensitization are associated with subsequent asthma development. We aimed to understand the consequences of respiratory syncytial virus (RSV) infection and/or house-dust mite (HDM) exposure on cytopathogenesis, virus replication, and innate immune responses in airway epithelium from young children.

Well-differentiated primary nasal epithelial cell (WD-PNEC) cultures were derived from children (aged 1-6 years) identified as (n=5 each):

1. Healthy
2. Mild wheezers
3. Severe wheezers

WD-PNECs were infected with RSV following HDM or mock stimulation. RSV growth kinetics were similar for all children. Post-infection ciliated cell loss was greatest from severe wheezer WD-PNECs. HDM pre-treatment did not alter innate immune responses to infection. Expression of *IL-29* and interferon-stimulated genes (ISGs), including *Ifi6, Isg15, Duoxa2, Duox2*, were significantly increased following infection, irrespective of wheeze status, as were IL-29, CEACAM1, TRAIL, CX3CL1, GM-CSF, CXCL8, and CXCL16 protein secretions. Interestingly, *Irf9*, *Ifi6*, and *Isg15* expression were significantly lower in mild compared to severe wheezers or healthy individuals. Furthermore, CEACAM1 and IL-33 secretions were highest, and GM-CSF was lowest, in WD-PNECs from severe wheezers. CXCL16 secretions were higher in wheezers compared to healthy children, irrespective of wheeze severity. Surprisingly, genes associated with asthma and airway remodeling, including *Tslp, Hmgb1, and Krt5*, were reduced following RSV infection in all cohorts.

Although differential gene/protein expression was observed in children with no, mild or severe wheeze, the functional consequences of these responses on the progression of wheeze pathogenesis remain to be determined.

A203

Investigating a Novel Packaging Mechanism by Staphylococcal Pathogenicity Island

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Abstract

Staphylococcus aureus pathogenicity islands (SaPIs) are phage satellites that exploit the life cycle of their 'helper' phages. Most SaPIs are packaged using a headful (pac) packaging mechanism with helper phage machinery. SaPIs interfere with *pac* phage reproduction through various strategies, including the redirection of phage capsid assembly to form small capsids that accommodate the smaller SaPI genome. This process typically depends on the expression of the SaPI-encoded *cpm*A and *cpm*B genes encoded in classical operon 1 of the SaPI genome. However, another SaPI subfamily, which includes SaPI1028, can remodel one of the helper phage capsids into a small capsid without encoding *cpm*AB homologs. Hence, the basis for this interference remains to be deciphered. Using techniques like DNA cloning, Southern blotting, and electron microscopy, I have identified and characterized a novel mechanism by which SaPIs manipulate the helper phage capsid. This process depends on a new SaPI-encoded gene, *rcp* (redirecting capsid packaging), which encodes a protein involved in remodelling the phage capsid into a small capsid to package the SaPI genome. It was shown using a BACTH assay that the Rcp targets the phage major capsid protein. As the protein sequences of Rcp and CpmAB are unrelated, this strategy represents a fascinating example of convergent evolution. This result, moreover, indicates that the production of SaPI-sized particles is a widespread strategy of phage interference conserved during SaPI evolution.

A204

Diversity of the Type VI Secretion System in the *Neisseria* spp.

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Abstract

Previously, we published the first description of complete Type VI Secretion Systems within the genome sequences of *N. subflava* isolates. Two different Type VI Secretion System types were identified and are here defined and characterised as T6SS-A and T6SS-B. Since publication of our first report, a full analysis of the presence and diversity of the Type VI Secretion System in *Neisseria* spp. has been undertaken. *Neisseria* spp. have now been identified with both T6SS-A and T6SS-B secretion system types as well as species with a subtype of the T6SS-B, defined here as T6SS-Bi. The characteristics of T6SS-A and T6SS-B in *N. subflava* and other *Neisseria* spp. are analysed in detail, including assessment of the lineages containing one or both of these types of T6SS. Phylogenetic analysis highlights the diversity of two types of Type VI Secretion Systems in *Neisseria* spp., with T6SS-A, T6SS-B, and T6SS-Bi overall clustering according to species. Evidence also supports horizontal gene transfer of T6SS core gene clusters between different *Neisseria* species.

A205

Genome analysis reveals the potential health risks conferred by an eggplant-associated *Pseudomonas aeruginosa* PPA14

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Abstract

Pseudomonas aeruginosa is a Gram-negative bacterium predominantly found in soil, water bodies, moist surfaces, plants, animals, and humans. Patients with chronic pulmonary diseases, medical implants, and weakened immune systems are highly prone to hospital-acquired *P. aeruginosa* infection. The agricultural ecosystem is one of the vast reservoirs of *P. aeruginosa*. However, there were limited attempts to analyze the health risks associated with plant-associated *P. aeruginosa* (PPA). We have previously assessed the resistome profiles of 18 *P. aeruginosa* strains isolated from rhizospheric and endophytic niches of edible vegetables. Among these, an eggplant rhizospheric strain PPA14 exhibited resistance against seven classes of antibiotics *in vitro*. In this study, we sequenced this strain using the Solexa-Illumina and Oxford-Nanopore platforms, assembled and annotated the complete genome. The ABRicate tool detected the presence of virulence-related and antibiotic resistance (ABR) genes and validated using multiple reference databases such as VFDB, NCBI AMRFinderPlus, MEGARes, CARD, and ResFinder. The location of genomic islands was detected using IslandViewer4. The PPA14 genome size was 6.72 Mbp, encoding 6322 open reading frames. The genome harbored 49 ABR genes, including those coding for multiple families of efflux pumps that collectively confer resistance against at least 11 classes of antibiotics. In addition, we detected 225 virulence-related genes and 83 genomic islands that were potentially acquired through horizontal gene transfer. Over 4% of the PPA14 genome is devoted to conferring virulence and extensive drug resistance. Our report highlights the health threat associated with the *P. aeruginosa* flourishing in the agricultural ecosystem.

A206

Phage-inducible chromosomal islands promote genetic variability by blocking phage reproduction and protecting transductants from phage lysis

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Abstract

Bacteria need to protect themselves from infection and killing by phages to survive in the environment. For this purpose, bacteria have developed a sophisticated arsenal of defense mechanisms that can protect individual cells or the overall bacterial population. Individualized protection is achieved via systems such as CRISPR-Cas or lysogenization that allow the infected cell to survive. By contrast, population-based protection such as abortive infection systems lead to the cell's death before phage progeny is released. Here we describe a new role for phage-inducible chromosomal islands (PICIs) in protecting individual cells and bacterial populations from phage predation. The resulting increased survival has consequences for the acquisition of foreign DNA such as antimicrobial resistance and fitness genes by the prey population as it also allows for the increased survival of bacteria that have acquired new genetic material. As a direct consequence of this increased survival, PICIs expand genetic diversity in bacterial populations. Such increased genetic diversity is advantageous to the complete bacterial population as the best adapted clones will outcompete others in any given environment. PICIs therefore also act as key mediators of population diversification.

A207

Identification of a novel cyclic-β-glucan synthase family in Pseudomonas

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Abstract

Bacteria produce different polysaccharides with a variety of functional roles. However, the identification of synthases is becoming increasingly reliant on homology with a relatively few well characterised enzymes. Recently, we began a bioinformatics investigation of an 'Orphan' bacterial cellulose synthase catalytic subunit (BcsA)-like protein found in three model pseudomonads, *P. fluorescens* SBW25, *P. putida* KT2440 and *P. syringae* DC3000. These Orphans were not associated with the fully functional *bcs* operons and pair-wise alignments indicated that they were not recent duplications of the operon gene, and with less than 41% identity were unlikely to have a similar function. We identified over 112 Orphan orthologues among the pseudomonads suggesting they may have some other adaptive role. Conserved domain and secondary structure predictions suggest a two-domain structure for the Orphan protein including a periplasmic β-glucanase domain and a cytoplasmic glycosyltransferase (GT)-like family domain with eight transmembrane helices, with conserved motifs and catalytic residues identified by comparison to enzymatically characterised orthologs. The *P. aeruginosa* PA14 Orphan (NdvB) is required for cyclic β-(1→3)-glucan (CβG) synthesis which is involved in biofilm-associated antibiotic resistance and suggest that the Orphans represent a larger family of CβG synthases. In other bacteria, CβGs are involved in osmoregulation, plant infection and induced systemic suppression, and might provide similar functions for pseudomonads. Our Covid-lockdown inspired bioinformatics research illustrates how important it is to critically evaluate automated functional annotations, and how many more uncharacterised polysaccharide synthases might be found in bacterial genomes.

A208

SENTINEL – SequENcing of hisTorical clINical *Escherichia coli* from the NEtherLands

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Abstract

Escherichia coli is an important opportunistic pathogen for understanding the evolution and dissemination of antimicrobial resistance (AMR) genes. This study investigated a unique historical *E. coli* strain collection from invasive disease patients collected nationwide by the Netherlands Reference Laboratory for Bacterial Meningitis between 1975-2021.

The population dynamics of 1,389 *E. coli* isolates from 1084 patients collected before 2015 (~95% from infants <1 year old) were investigated using whole genome sequencing and bioinformatic analyses, allowing insight into the emergence and dominance of particular lineages and examination of the ever-changing AMR landscape. Isolate sources were the bloodstream (n=771), cerebrospinal fluid (n=591) and other sites (n=27).

Results proved, on average, that the number of resistance genes per genome did not increase between 1975-2015. Isolates had a wide range of genotypic drug-resistant mechanisms to a variety of antimicrobial classes, but no specific increasing occurrence of AMR genes. Only 25% isolates carried Extended Spectrum Beta-Lactamase genes. A range of phylogroups causing infection were identified in every study year, the most predominant overall being B2 (n=957). Two cryptic clade isolates (III and V) causing infection were identified. ST95 (n=372) and ST567 (n=173) occurred most frequently. Pandemic lineages, including ST131 (n=21), ST1193 (n=1) and 648 (n=1) were identified.

This study has identified the presence and diversity of AMR genes and STs and should reveal further insights into the evolution and diversity of Dutch pathogenic *E. coli* isolates over several decades.

A209

Plasmids do not consistently stabilize cooperation across bacteria but may promote broad pathogen host-range

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Abstract

Horizontal gene transfer via plasmids could favour cooperation in bacteria, because transfer of a cooperative gene turns non-cooperative cheats into cooperators. This hypothesis has received support from theoretical, genomic and experimental analyses. In contrast, using a comparative genomics approach across 51 diverse bacterial species, we found that genes for extracellular proteins, which are likely to act as cooperative 'public goods', were not more likely to be carried on either: (1) plasmids compared to chromosomes; or (2) plasmids that transfer at higher rates. Our results were supported by theoretical modelling which showed that, while horizontal gene transfer of plasmids can help cooperative genes initially invade a population, it has less influence on the longer-term maintenance of cooperation. Instead, we found that genes for extracellular proteins were more likely to be on plasmids when they coded for pathogenic virulence traits, in pathogenic bacteria with a broad host-range.

A210

Building Machine Learning Host-Prediction Models of *Salmonella Typhimurium*

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Abstract

Salmonella enterica is a diverse pathogen with over 2600 serovars that differ in host-specificity. Generalist serovars like *S. typhimurium* infect a wide range of hosts, while host-specific serovars have a narrower range. The genetic factors underlying host specificity are complex, but the availability of thousands of sequences and advances in machine learning have made it possible to build host prediction models which would be invaluable in outbreak control.

Previous studies have already showcased the potential of using machine learning to examine *S. enterica* host specificity, so we focused on building a host-specificity prediction model based on a wide range of genomic features. We collected 3300 *S. Typhimurium* assembled sequences isolated from humans, swine, bovine and poultry from the USA. SNPs, protein variants, AMR profiles and intergenic regions were extracted from high-quality assemblies, filtered, and fed into RandomForest machine learning models. Reproducible pipelines for model creation and new sequence testing have been built Nextflow with Docker.

The models based on protein variants and intergenic sequences had a similar average accuracy, comparable with previous studies (80-90%). AMR profile models had the lowest predictive accuracy (60-80%) with great variation within hosts. Poultry predictions had the highest accuracy (>90%), whereas bovine and human predictions had the lowest, in line with our dataset's phylogeny, which includes a large distinct poultry clade, and 'mixed' clades of human and bovine. Intergenic and protein variant models also performed better than phylogeny-based predictions, though extracting disambiguating biological vs phylogenetic importance of important genomic features is still ongoing.

A211

Spotlight on the meta-community of the South Tyrolean cheese microbiome

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Abstract

The interest towards the microbial consortia that govern fermented foods is increasing because it helps to understand the entire process of food production, including the cheesemaking process. This study aimed to investigate the Alto-Adige's Italian cheeses (milk-cheese axis) microbiome, to define the societal organization of the cheesebiome, identifying all the microbial players (dominant, sub-dominant and satellites) that show resilience during the ripening process. The partial sequencing of the 16S rRNA of ca. 1100 isolates was carried out. These isolates were selected among the 11880 isolated from cow's milk, curd and cheese samples at different ripening collected from three local dairies. Among the selected isolates, 43 species were identified, mostly belonging to the genera *Lactobacillus, Lactococcus* and *Streptococcus*. Other species, rarely found in dairy products, were also identified such as *Acinetobacter johnsonii, Chryseobacterium aquaticum, Citrobacter amalonaticus, Klebsiella oxytoca, Escherichia coli, Hafnia paralvei, Serratia liquefaciens,* and *Corynebacterium flavescens*. Culture independent methods (library preparation and MiSeq Illumina analysis) allowed the investigation of the bacterial and yeast composition. One hundred and ninety-eight bacterial taxonomic groups were found at genus level where 148 of them were identified only in milk and/or starters. A total of 173 yeast taxonomic groups were detected at genus level and 123 of them were found only in milk or in natural starter composition. *Lactobacillus, Streptococcus, Debaryomyces* and *Nectriaceae* were the dominants genera from 1 month ripening onwards.

A212

A complete genome sequence for *Staphylococcus edaphicus*, strain CCM 8731.

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Abstract

Staphylococcus edaphicus, a member of the coagulase-negative group of the Gram-positive staphylococci, was recently isolated from Ross Island, Antarctica. Here, we present the first complete genome sequence for *S. edaphicus* strain CCM 8731 generated using an Illumina-Oxford Nanopore hybrid-assembly approach.

Briefly, *S. edaphicus* CCM 8731 was grown on trypticase-soy agar overnight to obtain single colonies, with a single colony transferred to 10 mL of brain-heart infusion broth and grown again overnight. High-molecular-weight genomic DNA was extracted using Qiagen 100/G gravity flow columns. Illumina library preparation was performed according to the standard Illumina Nextera XT protocol and sequenced on a MiSeq using v2 chemistry for 250 bp paired-end reads. Nanopore library preparation was performed using a modified native barcoding protocol, with the library loaded on a Flongle. Once the Nanopore data was assembled into one contiguous sequence the assembly was polished with the Illumina data and annotated using Prokka. The resulting assembly is 2,781,595 bp in size with a G+C content of 33.4% and a predicted 2776 genes, including the antibiotic resistance genes (ARGs) *fusD*, *blaZ* and *mecC* conveying resistance to fusidic acid and β-lactams respectively.

Complete genomes are often included in datasets to serve as accurate references for the species and strains used in the study. Due to *S. edaphicus*' evolution within an extreme environment while still retaining mobile genetic elements and ARGs observed in other staphylococcal species, such as its closest phylogenetic neighbour *S. saprophyticus*, *S. edaphicus* represents a significant species of interest for evolutionary comparisons with the genus.

A213

One shade of grey: characterisation of a novel colony variant of *Acinetobacter baumannii* AB5075

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Abstract

Acinetobacter baumannii is a critical priority pathogen for which new antibiotic treatments are urgently required. The commonly studied isolate AB5075 is well documented to undergo a high frequency phase switch between virulent opaque (VIR-O) and avirulent translucent (AV-T) colonies. Such colonies can only be distinguished using oblique illumination microscopy. We have observed colonies (here termed "grey" variants) that appear clearly translucent under room lighting, with a number of interesting phenotypes.

To investigate the transcriptome of VIR-O and grey variants of AB5075, we used the techniques of Cappable-seq, total RNA-seq and Term-seq which enabled genome-wide annotation of transcription start and stop sites. Comparative analysis of transcripts between VIR-O and grey variants shows significant upregulation of an IS5-like element ISAba13 family transposase. This is coupled with significant downregulation of an acetyltransferase and glycosyltransferases which form part of the capsule synthesis operon. Long read genome sequencing identified the insertion of an ISAba13 insertion sequence into this operon, effectively blocking transcription.

Phenotypic characterisation of grey variants shows they are more hydrophobic and have a greater ability to form biofilms. Furthermore, grey variants are less motile and have a reduced ability to resist aminoglycosides and undergo natural transformation. These findings highlight the potential to generate heterogeneity within bacterial populations through the use of mobile genetic elements, with important ramifications on the ability to cause disease and resist antibiotic treatment.

A214

Mechanisms for positive and negative regulation of helicase recruitment to a bacterial chromosome origin

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Abstract

Bidirectional DNA replication from a chromosome origin requires the loading of two helicases, one for each replisome. The molecular mechanism for helicase loading at bacterial chromosome origins is unclear. Here we report both a positive and a negative mechanism for directing helicase recruitment in the model organism *Bacillus subtilis*. Systematic characterization of DnaD revealed separate protein interfaces required for interacting with the master initiator DnaA and with a novel single-stranded DNA (ssDNA) sequence located in the chromosome origin (DnaD Recognition Element, "DRE"). We propose that the location of the DRE within the replication origin orchestrates strand-specific recruitment of helicase to achieve bidirectional DNA replication. We also report that the developmentally expressed repressor of DNA replication initiation, SirA, acts by blocking the interaction of DnaD with DnaA, thereby inhibiting helicase recruitment to the origin. These findings significantly advance our mechanistic understanding of helicase recruitment and regulation of DNA replication initiation at a bacterial chromosome origin.

A215

Using TraDIS to compare the probabilities of transposon insertion throughout the *Escherichia coli* genome.

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Abstract

Transposon directed insertion-site sequencing (TraDIS) is a very powerful, whole genome method that can be used to identify genotype-phenotype associations in bacteria. Once a transposon mutant collection has been made for an organism it can be used repeatedly to test different growth conditions.

Whilst the transposons used have a broad range of insertion sites, these are not random, leading to bias in the distribution of mutations. Sometimes such biases may result in a paucity of data for some genomic regions that are less favoured by the transposon.

This work describes a comparison of data from transposon mutant libraries in *E. coli* generated using two transposon types used commonly for mutagenesis; a Tn5-derivative and a *Mariner*-derivative with their respective transposases. For both, transposons were introduced into the parent strain using conjugation. The transposon insertion sites and number of mutants at each site were determined using a TraDIS-nucleotide sequencing variation of the Illumina platform. Three growth replicates were set up from three independent conjugations to produce nine libraries for each transposon.

With both biological and technical replicates, transposon insertion probability at each site in the genome can be modelled to refine the parameters used to class a gene as a candidate essential gene within analysis pipelines. Understanding the nuances of each transposase and accounting for biases in a seemingly 'random' mutagenesis system, allows us to perform more thorough analyses and obtain more representative candidate essential gene lists, in turn leading to better resolution of survival mechanisms under tested growth or stress conditions.

A216

FAIR Pathogen Data: Beyond Covid-19

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Abstract

The SARS-CoV-2 pandemic has brought microbial genomics and sequencing consortia to the forefront of public health. Never before has the concept of sequencing a pathogen routinely been so ubiquitous in the minds of the public or the scientific community. The European Nucleotide Archive (ENA) at EMBL-EBI, part of the INSDC, has been at the centre of archiving public sequencing data of various types for years and the SARS-CoV-2 pandemic is no exception.

The ENA has been the underlying database supporting the largest dataflows over the past two years in development of the European Covid-19 Data Platform (covid19dataportal.org), a portal interface linking a diverse range of biological computational data relevant to SARS-CoV-2. A platform for findable, accessible, interoperable and reusable (FAIR) Covid-19 related data. Over the course of the platform's existence there have been over 2.8 million raw read data sets archived and over 2.8 million sequences archived for SARS-CoV-2. Making SARS-CoV-2 read data sets now represents 16% of all raw read records in the ENA. Over the course of the pandemic many lessons on creating a platform for FAIR pathogen data have been learned.

Here I will present these lessons on building a platform for FAIR pathogen data for SARS-CoV-2 and how they are being applied to build a new platform for all pathogen data. One that will “FAIRify” more pathogen data enabling researchers around the globe to prepare for the next pandemic, and one which allows us to be ready to deploy elastic data services when that day comes.

A217

Cross-transmission of Feline Leukaemia Virus between domestic and wild felids in Chile

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Abstract

Feline Leukaemia Virus (FeLV) is gammaretrovirus of cats. FeLV is widespread among domestic cat populations and can infect many other felids where it represents a serious disease threat to several species of endangered felids. The main consequences of FeLV infection are hematopoietic disorders and neoplasia. Domestic cats have both endogenous (copies of virus in the cat genome) and exogenous variants of FeLV, these frequently recombine producing variants with recombinant envelope genes and alternate receptor usage. Until recently inter-cat transmission was presumed to be solely the exogenous (FeLV A) variant but recent studies in domestic cats and in wild pumas have demonstrated that the recombinant FeLV B variants are also transmitted horizontally. This is of concern in disease control as while effective vaccines for FeLV are in use, these are targeted against the FeLV A variant and may not be effective against other variants.

This research examined samples from Chilean felids including: free-range wild felids (*Leopardus guigna)*, felids held in zoological parks (*Caracal caracal*, *Leopardus guigna*, *Panthera leo*, *Panthera uncia*, *Leopardus pardalis*, *Panthera onca*, *Puma concolor* and *Panthera tigris*) and domestic cats (*Felis silvestris catus*). Samples had previously been determined to be positive for FeLV with an LTR end point PCR. This study will apply envelope gene PCR and NGS (Illumina) to determine the envelope gene diversity and transmission dynamics of FeLV variants in Chilean Felids. The primary aims of the study are to determine if FeLV B (or other envelope variants of FeLV) are being transmitted into the non-domestic populations.

A218

Reaching the sweet spot: Identification of a novel arabinose uptake locus in Enterohaemorrhagic *Escherichia coli*

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Abstract

Enterohaemorrhagic *Escherichia coli* (EHEC) is a major foodborne pathogen of the human colon. During infection, EHEC exploits the intestinal nutrient landscape by scavenging uncontested nutrients to avoid competition with the native gut microbiota. However, our understanding of how pathogens overcome nutritional constraints conferred by the microbiota is incomplete. EHEC relies on monosaccharides for energy due to an inability to degrade complex polysaccharides. Arabinose is an abundant monosaccharide in nature and can support EHEC growth as a sole carbon source. We have identified a novel locus predicted to encode a carbohydrate uptake transporter, located on a horizontally acquired O-island in EHEC. Gene expression analysis of the system identified a >300-fold increase in transcription exclusively in the presence of arabinose. Inspection of the promoter sequence revealed a potential AraC binding site, the transcriptional regulator of the canonical arabinose utilisation system in *E. coli*. Deletion of *araC* completely abolished transcription and could be complemented by expression of AraC *in trans*, indicating adapted regulation of this horizontally acquired locus by a native transcription factor. Phylogenomic analysis of ~1000 sequenced *E. coli* genomes revealed carriage of this locus predominantly by EHEC strains, suggesting potential importance to the pathotype. We propose that this additional arabinose system enhances the ability of EHEC to scavenge preferred nutrients *in vivo*, providing a competitive advantage during colonisation. Understanding the ability of EHEC to acquire nutrients is crucial for deciphering nutrition-related mechanisms of pathogenesis and could ultimately lead to the design of treatment strategies that prevent colonisation of the host.

A219

NDM-5 and OXA-181-producing *Escherichia coli* detected in treated and untreated UK wastewater

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Abstract

Antimicrobial resistance (AMR) is rapidly becoming one of the greatest threats to modern healthcare around the world. The numbers of resistant infections have been rising rapidly over the last few decades, and mobile genetic elements (MGEs) carrying genes that confer resistance to our most valuable antibiotics have played a major role. Antimicrobial resistant bacteria are frequently isolated from wastewater treatment plants, and identification of their associated resistance genes and MGEs can be used to help understand the landscape of AMR in a local environment. The work described in this paper investigated the presence of carbapenemase-producing Enterobacterales (CPE) in wastewater from the UK East Midlands. CPE were isolated from both treated and untreated wastewater, and whole genome sequencing was used to show that the same strain of NDM-5-producing *E. coli* could be detected in treated and untreated samples taken one week apart. Eight of ten sequenced isolates contained $bla_{\text{NDM-5}}$ within IS*26* arrays found on mobilisable IncF plasmids, with the remaining two harbouring chromosonally-encoded $bla_{\text{NDM-4}}$ and $bla_{\text{NDM-5}}$ respectively. Additionally, one isolate harboured $bla_{\text{OXA-181}}$ on an IncX3-ColKP3 fusion plasmid. All of the sequenced isolates contained multidrug-resistant plasmids with a high level of relatedness to those isolated from clinical samples around the world, suggesting these bacteria have the potential to cause severe and difficult to treat infections.

A220

Re-analysis of an outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 associated with raw drinking milk using Nanopore sequencing

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Abstract

Whole genome sequencing has revolutionised public health microbiology and it is now possible to sequence and generate single contiguous *de novo* assemblies of complex, prophage rich bacterial genomes, such Shiga toxin-producing *Escherichia coli* (STEC).

We used Oxford Nanopore Technology sequencing data to quantify genetic relatedness and characterise microevolutionary events in the accessory genomes to assess the within-outbreak variation of seventeen genetically and epidemiologically linked isolates related to consumption of raw drinking milk in 2017, and three closely related strains isolated in 2019.

Upon completion, nineteen of twenty samples had zero variants called between each respective sequencing technology. There was a 0.65Mbp large chromosomal inversion, within one sample relative to the remaining samples. Furthermore, we detected two types of the bacteriophage-encoded, highly pathogenic Shiga toxin variant, *Stx2a*; one typical of this lineage, the second was atypical and inserted into a site usually occupied by *Stx2c*-encoding bacteriophage. Acquisition of this second *stx2a*-encoding bacteriophage may be a factor in the enhanced symptom severity observed in cases infected with this strain. Finally, we observed an increase in the size of the pO157 IncFIB plasmid (1.6kbp) in isolates from 2017 compared to those from 2019, due to the duplication of insertion elements within the plasmids from the more recently isolated strains.

The ability to characterise the accessory genome in this way is the first step to understanding the significance of these microevolutionary events and their impact on the evolutionary history, virulence and potentially the likely source and transmission of this zoonotic, foodborne pathogen.

A221

Investigating the fundamental differences in the essentiality of cell wall synthesis in *B. pertussis.*

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Abstract

Bacterial growth and replication are dependent on cell wall synthesis producing and assembling the membrane components allowing the bacterium to successfully interact and tolerate the exterior milieu. Peptidoglycan is one such component, forming a sacculus between the inner and outer bacterial membranes, providing structural support facilitating tolerance of osmotic pressure. In gram-negative bacterium the peptidoglycan synthesis pathways are essential with gene knockout producing unviable mutants.

However, transposon directed insertion sequencing (TraDIS) displayed that the essentiality of peptidoglycan synthesis processes was dependent upon the activation state of the regulatory BvgAS two component system in *B. pertussis*. The *mre/mrd* operon was observed to be conditionally essential during plate growth, depending on the activation of the BvgAS two component system, but entirely non-essential during bacterial growth in liquid culture. This poses the question: how is a fastidious bacterium like *B. pertussis* tolerating the loss of these classically essential genes?

Through mutagenesis of the *mre/mrd* operon and RNAseq analysis of wildtype *B. pertussis* in plate and liquid growth mediums, we investigated the effect of gene knockout on bacterial morphology and viability in addition to interrogating the potential genetic factors that could be involved in the conditional tolerance of *mre/mrd* knockout.

Overall, this work reveals that the *mre/mrd* operon can be genetically manipulated in *B. pertussis* resulting in altered external and internal bacterial morphology without reducing bacterial viability. RNAseq analysis of wildtype gene expression during BVG+ and BVG- growth in plate and liquid culture highlighted potential genetic mechanisms of tolerance which will be elucidated in future work.

A222

Comparative Genomics of Specialised Metabolite Biosynthetic Gene Clusters in the Actinomycete Genus *Micromonospora*

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Abstract

The specialised metabolites produced by actinomycete genera, most famously *Streptomyces*, are of critical importance as antibiotics, immunosuppressants, and anti-cancer drugs. Despite this, there is a dearth of understanding as how the production of these metabolites has played a role in the evolution of actinomycete genomes.

We sought to answer this by comparing the chromosomal loci of specialised metabolite biosynthetic gene clusters (smBGCs) in high-quality whole genome assemblies of the actinomycete genus *Micromonospora*. By using antiSMASH and BiG-SCAPE to respectively identify and group BGCs, we fitted the location of smBGCs to circular *Micromonospora* chromosome architecture. We compared the loci of *Micromonospora* smBGCs to smBGCs in the linear chromosome of *Streptomyces* species and identifed a smBGC rich mid-chromosome region that was analogous to the smBGC rich arms of *Streptomyces*. We also uncovered that *Micromonospora* possess two distinct regions of the chromosomes enriched with smBGCs. The first region, flanking the origin of replication, contains a conserved suite of smBGCs in fixed locations. The second region, at the mid-chromosome, contains not only more smBGCs, but a more diverse repertoire of them that suggests a higher turnover of smBGCs occurs here.

This work helps us to understand actinomycete secondary metabolism and highlights the utility of high-quality genome sequence assemblies for investigating the evolution of bacterial chromosomes. It offers insights as to which metabolites may be useful to bacteria and thus evolutionarily fixed, and aids genome mining efforts by showing that not all regions of the chromosome are equal when it comes to specialised metabolism.

A223

CRISPR-Cas-mediated removal of AMR plasmids is limited by presence of toxin-antitoxin systems.

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Abstract

Plasmids are mobile genetic elements and essential components of microbial ecosystems. As they often carry antimicrobial resistance (AMR) genes, plasmids can be a major contributing factor to drug resistant infections. Removal of unwanted plasmids may provide a means of resensitising pathogens to antimicrobials.

Previously, we constructed a conjugative CRISPR-Cas9 plasmid which can transfer to target bacteria and remove resident plasmids; removal of synthetic plasmids was easily achieved. However, it is unclear if this CRISPR delivery tool can effectively target natural plasmids, which are typically larger and often encode multiple AMR genes, stability systems, and other payload genes.

Here, we constructed a series of target plasmids derived from multi-drug resistance plasmid RP4 and identified that toxin-antitoxin system presence protects from CRISPR-mediated removal. Despite RP4 removal not being effective, the CRISPR delivery tool was used to prevent uptake of this target plasmid in a natural isolate.

This study reveals that toxin-antitoxin systems on target plasmids can limit their removal by CRISPR delivery tools, which is valuable for informing applications of antimicrobial resensitisation using CRISPR-Cas9.

A224

The phylogenomic landscape of the genus *Serratia*

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Abstract

The genus *Serratia* has been studied for over a century and includes clinically-important and diverse environmental members, yet has remained remarkably understudied at a genomic level. Available sequence information is dominated by multidrug-resistant clinical isolates of *S. marcescens* and a comprehensive understanding of the population structure, evolution and genomic capacity of the genus, and the species within it, is lacking. We have addressed this by assembling and analysing a balanced set of 664 genomes from across the genus, including sequencing 215 isolates originally used in defining the genus. Phylogenomic analysis of the genus reveals a rich and clearly-defined population structure aligning with ecological niche, as well as striking congruence between historical biochemical phenotyping data and contemporary genomics data. We show, through detailed analysis of the pan-genome, metabolic pathways, GC content and plasmid portfolio, that whilst speciation and niche specialisation events can be observed, *Serratia* exhibits striking diversity and plasticity, with examples of 'plasticity zones' and flow of genes across the genus being evident. This work provides an essential platform for future studies of the emergence of clinical and other lineages of *Serratia*.

A225

Reclassifying *Campylobacter jejuni* CC-353 strains based on phylogeny and antibiotic resistance

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Abstract

Campylobacter is a foodborne bacterial pathogen that can cause gastroenteritis in humans. It is on the CDC 2019 AR Threats Report due to increasing antibiotic resistance. As a zoonotic organism, *Campylobacter* can be isolated from a broad range of animal reservoirs and infect humans *via* contaminated food, water, and milk. 90% of campylobacteriosis cases are attributed to *Campylobacter jejuni*. We identified one clonal complex (CC353), which displayed a sigmoidal increase in fluoroquinolone resistance between 1998 and 2018. Phylogenetic analysis of the sequence types (STs) identified five distinct sub-groups of CC353. The results suggest that the definition of CC353 requires revision. In addition, using ClonalFrameML analyses, we identified high levels of recombination within a sub-group of CC353.

Further investigation identifies that ST581, an ST within current definitions of CC353, displayed distinct patterns of fluoroquinolone resistance over time. Consequently, this has been classified as the central genotype of a new clonal complex, CC581, based on whole-genome analysis, the large number of STs and their biological properties. Two out of the four remaining subgroups were not sufficiently large to assign a new clonal complex, but the fourth and the fifth clusters potentially require a new central genotype to be identified. Our findings remind us that MLST schemes were established more than a decade ago with limited genomic data for many organisms. Whilst these nomenclatures have proven mainly robust in the era of next-generation sequencing, broader sampling regimes and the ever-increasing amount of whole-genome sequence data available can occasionally necessitate the re-classification of clonal complexes.

A226

The identification of six fluoroquinolone resistance patterns in *Campylobacter jejuni* clonal-complexes by longitudinal studies [1998-2018] in the UK

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Abstract

Campylobacter is a foodborne bacterial pathogen and can cause gastroenteritis in humans. 90% of campylobacteriosis cases are attributed to *Campylobacter jejuni*. *Campylobacter* is frequently exposed to antibiotics in animal production. Campylobacteriosis is usually a self-limiting disease, but antibiotics such as macrolides may be used in young, elderly, or immunocompromised patients. Fluoroquinolone resistance occurs spontaneously and is most associated with the Thr-86-Ile mutation in the *gyrA* gene. Resistant variants can quickly become dominant with no apparent fitness cost to the bacterium. Antibiotic-resistant strains threaten the food chain, including humans as the end consumer.

We determined *C. jejuni* fluoroquinolone resistance patterns amongst human disease isolates over 20 years. A total of 10,359 genomes from UK human stool isolates recorded in the PubMLST database (https://pubmlst.org/) between 1998 to 2018 were analysed. Fluoroquinolone resistance profiles were inferred from genotype data, as previous studies had shown that such predictions are 98% accurate. Linear regression analysis indicated that *C. jejuni* had become increasingly resistant to fluoroquinolones over time, as seen in numerous observational studies. From GLM models, six distinct fluoroquinolone resistance types were observed over time. Some *Campylobacter* variants showed only resistance, some had changed entirely from susceptible to resistance in a sigmoidal manner, and some remained susceptible over the observation period. These results indicate that the *C. jejuni* fluoroquinolone resistance phenotype is complex and should be analysed with respect to strain dynamics. Moreover, there is evidence of increasing fluoroquinolone resistance in some *C. jejuni* variants, despite the much-reduced use of these agents on UK farms.

A227

The diversity of the Stramenopile supergroup is reflected in the multiplicity of their B vitamin metabolism

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Abstract

The Stramenopiles are a hugely diverse group of organisms with varied lifestyles and habitats. Some members of the group, namely the diatoms, are very well studied but many other branches of the group remain elusive. Currently, the majority of the heterotrophic members of the Stramenopiles are unculturable. This emphasises the need to gain an understanding of their metabolism to inform future culturing efforts. Previous studies have demonstrated some of the photosynthetic members of the Stramenopiles are dependent on an external source of vitamin B12 for growth. Here we demonstrate with a comparative genomics approach that the trait of B12 auxotrophy is widespread across the heterotrophic members too. Evidence from the biosynthesis capabilities for other B vitamins suggests that these heterotrophic organisms may also be dependent on external sources of other B vitamins not required by diatoms. We also demonstrate that all members of the group appear to have the ability to biosynthesise the essential metabolic co-factor NAD+ but different biosynthesis pathways are utilised across the supergroup in a polyphyletic manner.

A228

Investigating evolutionary trends of *Escherichia coli* pangenomes

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Abstract

Escherichia coli is a diverse species spanning both common commensals of humans and animals and clinically important, major global pathogens. Pangenomics can facilitate the understanding of the diversity within this species. Previous work on ST131 found allelic diversity in metabolism and colonisation genes that was linked to multidrug resistance. To develop this work further, we have examined *E. coli* as a whole species on a lineage-by-lineage basis. 20,577 genomes were used to construct pangenomes for 21 distinct *E. coli* sequence types. We have identified sequence types enriched in metabolism genes, lineage-specific differences in core metabolic profiles, and lineage-specific alleles that may shed light on sequence type-specific metabolic adaptions that appear to evolve independently of phylogeny. These findings also provide evidence for differential evolution of metabolism genes. Our results suggest that selection and evolution of lineage-specific *E. coli* pangenomes may be happening in a non-phylogenetic manner and that sequence types are distinct in different ways. Further understanding of how these lineages differ will be key in future research to manipulate the abilities of specific *E. coli* lineages in their relevant environments.

A230

Local Point Prevalence Survey (PPS) captures hidden outbreaks of Carbapenemase-Producing Enterobacterales (CPE)

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Abstract

Background: A local PPS was performed to evaluate the existing CPE screening strategy. Whole-genome sequencing (WGS) was used to characterise circulating strains and determine the relatedness of CPE colonisation cases detected through the PPS and routine screening.

Methods: The PPS was performed in a London hospital group through rectal sampling of all inpatients in 2019. Swabs were cultured on selective agar and carbapenemase positivity determined by rapid EIA or PCR. Each CPE isolate detected in the PPS was matched to 3-4 contemporaneous routine screening isolates (same species and resistance mechanism). Following Illumina sequencing, genomic analysis included identification of acquired AMR genes and plasmids, genotyping and phylogenetic analysis.

Results: The PPS identified CPE isolates from 17 patients; 10 of which were newly identified cases. PPS isolates were enriched by matching to 59 screening isolates. 38 isolates carried $bla_{\text{OXA-48}}$, 12 $bla_{\text{NDM-1}}$, 6 $bla_{\text{NDM-5}}$, 6 $bla_{\text{OXA-181}}$, 5 $bla_{\text{OXA-232}}$, 4 $bla_{\text{VIM-1}}$, 3 $bla_{\text{IMP-70}}$ and 2 $bla_{\text{OXA-244}}$. 76% (29/38) $bla_{\text{OXA-48}}$ genes were encoded by a highly conserved IncL/M conjugative plasmid harbouring a variety of AMR genes in different Enterobacterales. The emergent $bla_{\text{VIM-1}}$ gene was encoded in another broad-host-range plasmid, IncN2, in closely related *K. pneumoniae*. Comparison of reconstructed plasmids with publicly available genomes provided an insight into the evolution of these plasmids.

Conclusion: Our PPS detected multiple new cases of CPE colonisation. WGS revealed an unsuspected cluster of $bla_{\text{VIM-1}}$-producing *K. pneumoniae* and found a possible interspecies $bla_{\text{OXA-48}}^{+}$ plasmid outbreak. This study highlights the utility of WGS for local surveillance to understand CPE emergence, persistence and transmission.

A231

Emergence of two distinctive multidrug resistant clones of *Corynebacterium striatum* among patients in intensive care unit during first wave of COVID-19 pandemic in the UK

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Abstract

Background

Corynebacterium striatum is part of normal skin and mucosal microbiota. Though rarely reported as a cause of nosocomial infections and outbreaks, in recent years specific multidrug resistant (MDR) *C. striatum* clones have emerged in healthcare settings. During April-July 2020 we observed an excess of *C. striatum* yielded from clinical samples with a MDR phenotype affecting critical care patients across three separate sites.

Materials

All isolates underwent phenotypic characterisation and typing by PFGE. Additionally, whole-genome sequencing was performed on 20 *C. striatum* clinical isolates obtained from intensive care patients and 10 contemporaneous clinical isolates. Genomic analyses included phylogenetic analysis, comparison to global *C. striatum* genomes and interrogation for presences of acquired resistance genes/plasmids.

Results

Two PFGE pulsotypes (cs2 and cs3) were identified, later confirmed by genomic analyses as two distinct clades of MDR *C. striatum* isolates. Within clades isolates were distinguishable by 0-14SNPs or 2-53SNPs, respectively. Between clade genomic variation was >20,000 SNPs. Both clones were seen in at least one other trust outside Greater London suggesting potential regional dissemination.

All *C. striatum* cluster isolates (n=20) carried genes conferring resistance to tetracyclines, aminoglycosides, macrolides. Clades contained specific recombination events and clade distinctive antimicrobial resistance genes: cs2 isolates carried genes conferring resistance to chloramphenicol, while cs3 isolates carried genes conferring resistance to sulfonamides.

Conclusions

We have characterised MDR *C. striatum* strains that emerged in intensive care patients during the first COVID-19 wave in the UK. National surveillance has subsequently commenced to further characterise *C. striatum* isolates across different hospital networks nationally.

A232

Understanding the Role of Replicon Linearity in the Dynamic Genome of *Streptomyces clavuligerus* for Improved Clavulanic Acid Production

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Abstract

To understand the complex genome of *Streptomyces clavuligerus (Sclav)*, the commercial producer of clavulanic acid, we elucidated the mechanism by which the ends of its five linear replicons are maintained (four linear plasmids (GLPs) and chromosome). Copies of *tap and tpg*, found on three of four GLPs, are essential for the maintenance of Streptomycete archetypal replicons and are required for replicon end-patching. We investigated plasmid-chromosome interactions to determine the role of Tap-Tpg in end patching of different replicons to cure GLPs for decreased metabolic burden and increased clavulanic acid production.

Previous work demonstrated *Sclav* chromosome circularisation and plasmid curing after cutting the largest GLP, pSCL4; potentially due to the loss of *tap-tpg*$_4$. To determine the role of *tap-tpg* in chromosomal/plasmid linearity, we tested inactivation *tap-tpgs* using CRISPR-dCas9 multiplexing; targeting *tap-tpg*$_4$ on pSCL4, *tap-tpg*$_3$ on pSCL3 and *tap-tpg*$_2$ on pSCL2. The abolition of transcription of *tap-tpg* pairs led to replicon circularity and plasmid loss. This suggests that end-patching in *Sclav* is carried out by a combination of Tap-Tpg pairs that operate both *in cis* and *in trans* on multiple replicons of this organism. Our current work focuses on plasmid and telomere loss via genome sequencing and Pulse Field Gel Electrophoresis.

Future work will focus on pSCL3 and pSCL2 Tap-Tpg interaction with pSCL4 and the chromosome after knockdown of the genes through sgRNA multiplexing. In this way, we will elucidate the *in trans* mechanisms of telomere replication and its effect on the dynamic genome of this commercially important organism.

A233

Widespread divergent transcription arising from bidirectional promoters in prokaryotes

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Abstract

Promoters are DNA sequences that stimulate the initiation of RNA synthesis. Bacterial promoters typically contain an AT-rich -10 promoter element, alongside additional sequences such as the -35 element. The arrangement of these elements dictates in which orientation RNA polymerase will bind to DNA. Thus, it has long been assumed that promoters in bacteria are directional.

We initially identified several promoters in *E. coli* where the direction of transcription was unclear. Many of these promoters were able to drive transcription in both directions; we define these as bidirectional promoters. A genome-wide analysis revealed that promoters in *E. coli* are frequently bidirectional. Mechanistically, this occurs because the -10 element has inherent symmetry and often overlaps on opposite DNA strands. Reciprocal stimulation between divergent transcription start sites also contributes. Evidence of bidirectional promoters is observed in multiple diverse prokaryotic species, including examples from Archaea. This suggests that bidirectionality is a common feature of prokaryotic promoters, which has important implications for understanding gene regulation.

A234

48-plex Long-Read Sequencing to Generate Complete Assemblies and Determine Genome Structure in *Salmonella* Agona

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Abstract

Background: Short-read sequencing, alongside multiplexing, has provided the resolution and high-throughput required to regularly identify SNPs in bacterial species important in human health. Such small nucleotide-level variations can have huge effects, from changing antibiotic resistance to altering entire metabolic pathways. Bacteria can also exhibit genomic variations, where large genome fragments shift position and/or orientation around long-repeat sequences to produce different unique genome structures (GSs) without necessarily affecting the underlying nucleotide sequence. Such variation cannot be identified by short-read sequencing as long-repeat sequences require reads of thousands of base pairs for resolution. Here we present the use of multiplexed, long-read sequencing to investigate GS relationships between strains of *Salmonella* Agona associated with acute and chronic infection.

Methods: A review of 2,233 *S*. Agona isolates from infections in England (2004-2020) and associated carriage was undertaken, in which 1,155 had short-read sequencing data available. A subset of 208 isolates was selected for high molecular weight DNA extraction and MinION long-read sequencing using a 48-plex, native ligation method, and GS analysis using Socru. Sequence data were also interrogated for AMR determinants, plasmid presence and phylogenetic relationships.

Results: 48-plex sequencing allowed successful generation of complete assemblies for samples with ~30X theoretical coverage. GS analysis revealed mostly the conserved arrangement GS1.0 and an unusual, imbalanced GS25.113 in a carriage isolate, where the origin and terminus were much closer together than in GS1.0, producing one very short and one very long replichore.

Conclusion: 48-plex of *Salmonella* genomes improves affordability of long-read, MinION sequencing via higher throughput.

A235

Determining prophage genome integration under single and co-infection: the case of Pseudomonas aeruginosa LES strains and their prophages in P. aeruginosa PAO1

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Abstract

Pseudomonas aeruginosa is an important opportunistic pathogen, causing nosocomial infections. The Liverpool Epidemic Strain (LES) is a major cause of mortality and morbidity in cystic fibrosis patients and harbours five prophages associated with increased fitness and survival in models of infection. However, little is known about how the co-habiting LES prophages influence the success of their bacterial host or how they interact with each other. Our study aims to better characterise LES prophage gene function and identify the molecular mechanisms by which they impact the biology of the P. aeruginosa host. We re-annotated the genomes of three LES prophages (Φ2, Φ3 and Φ4) following lysogenic infection of the well-characterised P. aeruginosa strain PAO1 to create single, double and triple lysogen variants harbouring all combinations of prophages. Manual curation revealed putative functions of previously unidentified ORFs. We also determined the integration sites of each LES prophage when carried alone or in combination with others in the model host PAO1 genome. While prophage Φ4 tended to target the host genome at random coordinates due to the transposase activity, both prophages Φ2 and Φ3 integrated at specific regions of the host genome. Interestingly, the integration regions of both prophages Φ2 and Φ3 are very close when they co-infect the host together, leading to chromosomal rearrangements and the loss of a ribosomal gene cluster. These data are crucial for unveiling the vast dark matter of temperate phages and enhancing our understanding of how bacterial and prophage genomes co-evolve.

A236

The global emergence of multidrug resistant *Campylobacter* lineages

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Abstract

The rise of antimicrobial resistance (AMR) in bacterial pathogens is widely acknowledged as one of the most serious threats to human and animal health, with some bacteria resistant to all known antibiotics. Diarrhoeal disease remains a major cause of child morbidity, growth faltering and mortality in low- and middle-income countries (LMICs), with *Campylobacter* among the most common causes. As a result of widespread resistance, *Campylobacter* is listed as a high priority pathogen by the World Health Organisation. Several studies have investigated the population structure and AMR in *Campylobacter* in developed countries, but little is known in LMICs where antimicrobial misuse is common. In this project, we analysed the genome sequence of >4,000 *C. jejuni* and > 1,000 *C. coli* human clinical samples isolated from >30 countries. We used the hierarchical BAPS algorithm to assign isolates into groups and screened all genomes against publicly available AMR databases. We identified novel lineages and high antimicrobial resistance in isolates from LMICs. High levels of multidrug resistance were detected in *C. coli* isolates globally and in some countries, isolates were resistant to up to six different antibiotic classes. Using a global outlook, we have identified emerging MDR lineages and assessed the risk of the developing AMR worldwide. As we begin to understand the relationship between antibiotic usage and AMR emergence, we can model AMR transmission networks for appropriate intervention strategies.

A237

Expansion and diversity of non-core protein modules in the *Potyviridae* evolutionary radiation

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Abstract

Potyviridae, the largest family of RNA viruses (realm *Riboviria*), represents one of the most notable evolutionary radiations among viruses (Gibbs *et al.*, Viruses 2020;12(2):132. doi: 10.3390/v12020132). The family has >200 species infecting plants and assigned to twelve genera within the phylum *Pisuviricota*, formerly known as the picorna-like supergroup (Dolja *et al.*, Annu Rev Phytopathol. 2020;58:23-53. doi: 10.1146/annurev-phyto-030320-041346). Their genomes are translated into polyproteins, which are hydrolyzed to mature, functional products.

Even though potyvirids have been studied for more than a century, the introduction of high throughput sequencing has revealed significant genomic variability and diversity of the protein modules. Genera of the family have a common polyprotein core which is expanded by a heterogeneous array of non-core modules.

Here we present an in-depth pan-family survey of the heterogeneity of non-core modules that expand the structural and functional diversity of the potyvirid proteomes. We provide a thorough family-wide inventory of the P1 and HCpro leader proteinases as well as other non-core protein modules including alkylation B (AlkB), pretty interesting sweet potato potyviral ORF (PISPO), inosine triphosphate pyrophosphatase (ITPase/HAM1) and pseudo tobacco mosaic virus-like coat protein (TMV-like CP). Finally, functional connections between non-core modules are highlighted to support host niche adaptation and immune evasion as main drivers of the family's evolutionary radiation.

A238

Gene-gene associations in pangenomes and human-associated microbial communities

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Abstract

Pangenomes describe the genes (core and accessory) present in a set of evolutionary related strains. This strain-to-strain variability can have important implications on antimicrobial resistance, virulence, and mechanisms of self-defense. We hypothesize that the variability of gene content in pangenomes is maintained by selection and that gene gain and loss is not random but instead reflects functional patterns within the genome. If this is the case, we might expect sets of genes to be gained or lost together across a pangenome which would manifest as patterns of gene-gene co-occurrence (i.e., association) and avoidance (i.e., dissociation). To test this hypothesis, we developed software (Coinfinder) which can detect genes that are present together more often than is expected by chance across a pangenome. We used Coinfinder to examine 209 *Pseudomonas sp.* genomes in which we found that the majority (86.7%) of abundant accessory genes form statistically significant co-occurrence and avoidance patterns. When compared to randomly paired genes, co-occurring genes shared more functionality, were more often expressed together, and are more likely to produce proteins that interact with each other. Taken together, these findings indicate that the accessory gene content of pangenomes are structured under the constraints of natural selection. We can next expand this idea to ask whether particular gene sets in the pangenomes of different members of a microbial community are more likely to be present together then would be expected by chance. Preliminary analyses suggest that this is indeed the case, indicating an underlying evolutionary-driven structure to human-associated microbial communities.

A239

Linking antimicrobial resistance genes to their bacterial hosts in the human gut microbiome using Hi-C

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Abstract

The complex microbial ecosystem inhabiting the human gut, termed the gut microbiome, can act as a reservoir for antimicrobial resistance genes (ARGs), collectively known as the gut resistome. Various methods have been developed to study the resistome, including using proximity ligation techniques, such as Hi-C, to link bacterial genes to phylogenetic markers, allowing ARGs to be linked to their bacterial host.

Here, we implemented Hi-C to investigate the bacterial hosts of ARGs in 4 human faecal samples. Using binning techniques on metagenomic assemblies from these samples, combined with Hi-C data, we were able to link 87 ARGs to their hosts across the 4 samples, out of a total of 119 ARGs identified by shotgun sequencing. ARGs carried on plasmids in an *Acinetobacter pittii* strain that was used as a spike-in were correctly linked to their host in all samples. We found that Hi-C was able to link ARGs to multiple contigs in each metagenomic assembly, and the main limiting factor in identifying the bacterial hosts of the ARGs was the success of the binning process and the ability to taxonomically classify the bins.

Following Hi-C analysis, the hosts of several ARGs were successfully cultured. Genome sequencing of these strains is ongoing, and these data will be used to validate Hi-C, and to further investigate the genomic context of the ARGs. Our data highlight the importance of gut commensals as reservoirs of antibiotic resistance genes.

A240

Streptococcus equi subsp. equi whole genome sequencing illustrates the intermediate steps involved in it's evolution from Streptococcus equi subsp. zooepidemicus

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Abstract

Background:

Streptococcus equi subsp. *equi* causes the equine respiratory disease 'strangles', which is highly contagious, debilitating, and costly to the equine industry. *S. equi* descends from the ancestral *Streptococcus equi* subsp. *zooepidemicus* and continues to evolve and disseminate globally. The acquisition of prophages and superantigen genes are key events in the pathogenic specialization of *S. equi*. Recent data from China may provide further insight into the transition of *S. equi* from *S. zooepidemicus* via an 'intermediary' clade.

Methods: 1141 isolates of *S. equi* were recovered from clinical samples submitted to collaborators and whole genome sequenced. Following creation of a phylogeny the collection was partitioned into clusters using Bayesian analysis of population structure (fastBAPS). Bayesian Evolutionary Analysis (BEAST) was used to estimate divergence of the Chinese samples. A pan-genome approach was used to compare the gene complement between clusters.

Results: We identified nine clusters of which 11/12 Chinese isolates belonged to a distinct, distantly related cluster (FB9). All FB9 isolates originated from donkeys rather than horses. Pan-genome analysis indicated that FB9 contained at least 20 genes absent from the rest of the collection and lacked genes found in all other clusters such as some related to iron transportation.

Conclusion: Initial data demonstrates that the FB9 cluster is an atypical *S. equi* lineage, characterised by differing gene content. This cluster of isolates will provide further insight into the specialization of *S. equi* into an equine specific pathogen.

A241

Investigating the regulation of the nisin O biosynthetic cluster in the human gut bacteria, *Blautia obeum*

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Abstract

Nisins are a family of antimicrobial peptides produced by some Gram-positive bacteria. Biosynthetic gene clusters (BGCs) contain genes required for nisin biosynthesis, regulation and immunity. Nisin O is produced by *Blautia obeum* and uniquely contains two two-component regulatory systems (NsoR1K1 and NsoR2K2) in its BGC. As nisin O has shown activity against both *Clostridium difficile* and *Clostridium perfringens* it is important to understand the regulation of the cluster. This will provide information regarding nisin O's role in potentially modulating the gut microbiome.

To test the function of these regulatory systems and their interaction with four predicted promoters previously identified within the BCG, a peptidase I (PepI) gene reporter assay was used with the promoters preceding *pepI* with constitutively expressed *nsor1k1* or *nsor2k2*. High levels of PepI activity were observed with the promoter preceding *nsor2k2,* which indicated constitutive expression. There was limited activity with the remaining three promoters. This indicated that there was no interaction between the regulatory systems and these promoters. To investigate whether the two regulatory systems were always present in close relatives of the nisin O BGC, 2094 *Lachnospiraceae* genomes from the Genome Taxonomy Database were screened; this identified six nisin O-like BCGs (between 83.1% to 98.8% base pair similarity), each containing the two regulatory systems. Screening these new clusters identified the presence of a new predicted promoter preceding *r1k1*.

A242

Genome sequence-based detection and analysis of plasmids for selected Enterobacteriaceae strains held by the UK's National Collection of Type Cultures

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Abstract

The National Collection of Type Cultures (NCTC) is a unique resource, composed of almost 6,000 medically relevant bacterial strains, maintained and developed by the UK Health Security Agency (UKHSA). The genomes of approximately 3,000 NCTC strains were recently long-read sequenced, with the genomic reads, assemblies and annotations uploaded to the European Nucleotide Archive (ENA) database under BioProject PRJEB6403. This wealth of genomic data gives us an unparalleled opportunity to assess the plasmid content of the sequenced strains, for many of which this information is not currently definitively known.

Here, we utilise a series of bioinformatic tools and approaches to identify and classify contigs of plasmid provenance from select NCTC whole genome assemblies, specifically those derived from strains belonging to Escherichia coli and Klebsiella pneumoniae species. Genome assembly optimization (e.g. adding short read information) was performed to investigate whether the current genomic assemblies adequately reflect the true plasmid content of the strains in question, and to assess whether plasmid sequences are captured accurately, in their entirety, and are circularisable. Work was also carried out to assess the AMR gene content of these plasmids.

The resulting dataset details the most comprehensive description of the plasmid population of the Escherichia coli and Klebsiella pneumoniae strains held by the NCTC carried out to date, revealing the NCTC strain set to be a rich source of diverse plasmids, containing both previously described and putatively novel structures.

A243

Geographical and temporal distribution of multidrug-resistant *Salmonella* Infantis in Europe and the Americas

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Abstract

Recently emerged *S.* Infantis strains carrying resistance to several commonly used antimicrobials have been reported from different parts of the globe, causing human cases of salmonellosis and with occurrence reported predominantly in broiler chickens. We performed core-genome based population structure analysis of 417 *S.* Infantis originating from multiple European countries and from the Americas. One hundred and seventy-one of the analysed isolates were collected from 56 distinct premises located in England and Wales (E/W), including isolates linked to incursions of multidrug-resistant strains from Europe associated with contaminated poultry meat, which facilitated comparison of isolates from different E/W sources with isolates originating from other countries. Maximum-likelihood and time-measured phylogenies, as well as Bayesian hierarchical clustering analysis, revealed that the E/W and central European isolates formed several disparate groups, which were distinct from the cluster relating to the United States and Ecuador/Peru, but the Brazilian isolates were closely related to the E/W and the central European isolates. Nearly half of the analysed strains harboured the IncFIB(pN55391) replicon typical of the 'parasitic' pESI-like megaplasmid found in diverse strains of *S.* Infantis. The isolates that contained the IncFIB(pN55391) replicon clustered together, despite originating from different parts of the globe. Most of the antimicrobial resistance (AMR) genes were identified in isolates that harboured IncFIB(pN55391) and/or one of the other detected 15 replicon types, and the long-read, but not the short-read, sequencing technology unequivocally confirmed that the identified AMR genes were associated with IncFIB(pN55391) and other detected plasmid replicon types.

A244

Understanding antibiotic resistance in *C. difficile*: an evolutionary approach

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Abstract

Clostridioides difficile is the most common cause of hospital-associated diarrhoea, and is associated with high levels of recurrence, morbidity and mortality – making it one of the most urgent threats to global healthcare. Resistance to many common antimicrobials is widespread, meaning treatment largely relies upon just three antibiotics - metronidazole, vancomycin and fidaxomicin. Since the recent discovery of pCD-METRO, conferring plasmid-mediated metronidazole resistance, the issue of antibiotic resistance in *C. difficile* has become increasingly pressing. Despite rapid advancement in available molecular biology tools, studying resistance in *C. difficile* using experimental evolution has remained an undervalued approach. Insights beyond first-step resistance, accumulation of mutations, population dynamics and alternative resistance pathways have thus far been chronically understudied. This project aims to utilise the huge technological advancements in genomic sequencing to genetically characterise the evolution of vancomycin resistance in *C. difficile.* Here, a multi-line directed evolution of engineered non-pathogenic clinically-relevant R20291 lines was performed using a gradient approach. 10 parallel lines were evolved over a period of 30 passages. 5 lines were hyper mutators, to obtain a wider view of resistance pathways. Resistance was observed to reach up to 32x MIC. Subsequent MICs of individual isolates confirmed mixed levels of resistance in the population. One isolate from each line with the highest end-point MIC was selected for illumina sequencing, providing insights into the targets and resistance pathways of this current front-line antibiotic. This method is combined with multiple-time-point 250x population sequencing, in an interdisciplinary approach to unpick the complex evolutionary dynamics involved in resistance.

A245

Phylogenetic and phenotypic analyses of a collection of food and clinical Listeria monocytogenes isolates reveals loss-of-function of Sigma B from several clonal complexes

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Abstract

To understand the molecular mechanisms that contribute to the stress responses of the important food-borne pathogen *L. monocytogenes*, we collected and whole genome sequenced a collection of 139 strains (Meat n = 25, Dairy n = 10, Vegetable n = 8, Seafood n = 14, Mixed food n = 4, and Food processing environments n = 78) mostly isolated in Ireland. These strains were compared to 25 Irish clinical isolates and 4 well-studied reference strains. Core-genome and pan-genome analysis confirmed a highly clonal and deeply branched population structure. Multi-Locus sequence typing showed that this collection contained a diverse range of strains from *L. monocytogenes* lineages I and II. Several groups of isolates with highly similar genome content were traced to single or multiple food business operators, providing evidence of strain persistence or prevalence, respectively. Phenotypic screening assays for tolerance to salt stress and resistance to acid stress revealed variants within several clonal complexes that were phenotypically distinct. Five of these phenotypic outliers were found to carry mutations in the *sigB* operon, which encodes the stress inducible sigma factor Sigma B. Transcriptional analysis confirmed that three strains carried mutations in *sigB, rsbV* or *rsbU*, predicted to result in loss-of-function of SigB. These strains exhibited increased tolerance to salt stress, and displayed increased sensitivity to low pH stress. Overall the study has shown that loss of function mutations in the *sigB* operon are comparatively common in wild-isolates, probably reflecting the cost of the general stress response to reproductive fitness in this pathogen.

A246

Construction and genomic characterisation of vacA allelic variants of the mouse-colonising Helicobacter pylori strain PMSS1

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Abstract

Helicobacter pylori (Hp) infection prevalence has declined over recent decades, whilst autoimmune diseases have become more common. Several groups report a protective link between *Hp* infection and multiple sclerosis (MS), and evidence suggests the VacA toxin plays a role in this. To identify which VacA polymorphic types are involved, we aimed to create *vacA* isogenic mutants for use in *in vitro* and *in vivo* models.

The *vacA* gene of strain PMSS1 (wild-type (WT) s2i2 *vacA*) was mutated to create variants with more active s1i1 and s1i2 *vacA* types. As PMSS1 has an active *cag* pathogenicity island (*cag*PAI), we also constructed *vacA* variants in a *cagE* null background to study toxin effects in the presence and absence of functional *cag*PAI. Mutations were confirmed by PCR and Sanger sequencing, prior to whole genome MiSeq sequencing.

Between 1 to 6 unexpected nonsynonymous mutations were found within the PMSS1 WT and six *vacA* mutant genomes. All strains possessed a single nucleotide difference from the published PMSS1 sequence within the *fur* (ferric uptake regulation) gene. This polymorphism was found in at least 6 other wild-type *Hp* strains. Five mutants contained a mutation within *rpoA* (RNA polymerase subunit alpha), but protein structural modelling software suggested this amino acid change is not within an essential protein domain, and would not alter the predicted secondary structure.

Relatively few secondary mutations were found within the wild-type and *vacA* variant strains. The constructed mutants are therefore suitable for future mechanistic studies of their impact on MS *in vivo*.

A247

Novel Metabolic Genes and an Altered Phasome in Campylobacter hepaticus Genomes are Potential Determinants of Spotty Liver Disease

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Abstract

Spotty liver disease is endemic to free range poultry and is characterised by low egg yield and increased mortality rates. In 2015, Crawshaw and colleagues attributed this disease to a novel Campylobacter sub-species Campylobacter hepaticus . C. hepaticus is able to readily colonise the liver of chickens whereas the closely related species, Camplyobacter jejuni, mainly colonises the gastrointestinal tract and only occasionally spreads to the liver and spleen of poultry. The mechanism of infection and niche liver occupation of C. hepaticus remains unknown. Using a range of in silico techniques we investigated reported gene loss in C. hepaticus and comment on possible gene function gains of relevance to niche liver occupation. We quantified variation in core and pan genome size and content across a wide selection of C. hepaticus isolates from poultry sources in the United Kingdom and Australia. Finally, we considered phase variation as a key influencer of niche environmental adaption, identifying novel short sequence repeat tracts in C. hepaticus by comparing its phasome to that of C. jejuni. Novel core genes and phasome may enable C. hepaticus to adapt to different ecological niches to C. jejuni and specfically faciltate profuse proliferation of C. hepaticus in the livers of chickens.

A248

An analysis of codon usage variation in Venezuelan equine encephalitis virus utilizing RNA-dependent RNA polymerase mutants

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Abstract

Venezuelan equine encephalitis (VEE) is a mosquito borne disease that infects small rodents and *Culex taeniopus* mosquitoes. Previous work has identified how intra-host diversity results in successful infection, and any change in the mutation frequency and/or profile results in attenuation during in vivo infections. One theory as to why altering the mutation frequency results in attenuation is that the changes in particular mutations alter synonymous codon usage reducing the optimisation of the virus. To test this, we used existing data sets from previous work using the 68U201 strain of VEE. A set of next generation sequencing data collected from both in vitro and in vivo infections of the virus 68U201 was analysed as a control. This included data from mosquito infections at multiple timepoints and in multiple tissues. To test if the attenuation is a result of changes in the mutational frequency, we tested three RdRp mutants that have been previously identified as having alterations in the frequency of mutations that results in significantly different mutational profiles. Here we identify variation and present an analysis of changes in codon bias occurring during these infections.

A249

Impact of multiple antibiotic resistance activator protein on bacterial flagellum biogenesis in Escherichia coli

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Abstract

Multiple antibiotic resistance is one of the main threats to human health in the modern world, compounded by insufficient development of new antibiotics. Multiple antibiotic resistance (*mar*) locus provides resistance to a wide range of antibiotics and phenolic compounds in gram-negative bacteria *E. coli.* The transcription factor MarA encoded in this locus is mainly required for activation and expression of the *acrAB-tolC* efflux pump, which plays the main role in active antibiotic transport out of the cell. However, MarA binds and activates many targets all over the bacterial chromosome which makes an even more significant contribution to the development of a resistant phenotype. In this work, we found new genes targeted by MarA that are involved in flagellar biosynthesis.

A250

The extensive taxonomic and functional diversity of the Ethiopian village chicken microbiota

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Abstract

Our understanding of the chicken microbiota has been greatly expanded due to studies using metabarcoding, culturomics, and metagenomics. However, most research has focused on commercial chickens raised in biosecure facilities; it is therefore likely that much microbial diversity has been missed, as free-range birds are known to have more diverse microbiota than conventionally housed birds.

In this study we characterised the microbiota of Ethiopian village chickens using metagenomic sequencing. These village chickens had predominantly indigenous genotypes, uncontrolled breeding, natural hatching, and a diverse diet from scavenging supplemented with limited crop residues. Shotgun sequencing was performed on DNA from 240 caecal samples from 26 villages in 15 districts. Short reads were assembled into contigs and then binned into putative genomes, before dereplication and quality control steps were performed to produce high-quality microbial metagenome assembled genomes (MAGs).

We constructed 9,977 strain-level MAGs and 1,790 species-level MAGs, representing diverse taxonomies, including 22 phyla from the Archaea and bacteria. We discovered 9682 strains, 1242 species, and 84 genera that were not present in previous chicken microbial genome datasets. These genomes encoded diverse carbohydrate degrading enzymes and metabolic pathways for the fermentation of various forms of fibre, highlighting their importance in fermenting indigestible carbohydrates. Pathways for methanogenesis, nitrogen, and sulphur metabolisms were also identified in these genomes.

Our findings demonstrate the richness of the microbiota in Ethiopian village chickens, highlighting the potential for further diversity discovery from rural chickens of other regions.

A251

Genomic and population epidemiology of the re-emerging *Shigella flexneri* 3a serotype in England, United Kingdom

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Abstract

Shigellosis is a sexually transmissible enteric infection caused by *Shigella* bacteria. Between 2009 and 2014, *Shigella flexneri* (*S. flexneri*) 3a was the most dominant species and serotype of sexually transmissible shigellosis among men who have sex with men (MSM) in England, United Kingdom. Subsequently, *S. flexneri* 3a diagnoses declined to pre-epidemic levels. However, in 2020, despite lockdown restrictions, *S. flexneri* 3a re-emerged as the dominant species/serotype associated with sexual transmission. Through a combination of whole-genome-sequencing and population epidemiological methods, our work aimed to elucidate the reason(s) for the re-emergence of *S. flexneri* 3a, be those genomic or environmental changes, or changes in the behaviour and immunology of the host. The age and geographical distribution of cases in the 2009-2014 cohort compared to the 2018-2020 cohort is largely unchanged, with the majority of cases among those aged 35 - 64 years, in London and the Southeast of England. Enhanced surveillance questionnaires (ESQ) were available for 67/177 (37.9%) cases in 2019-2020, and 24/177 (35.8%) reported being hospitalised, with a median admission length of 2 days. The majority (n=36, 53.7%) self-identified as a gay or bisexual man. Phylogenetic analysis showed most strains driving the re-emergence had evolved from the 2009-2013 epidemic strains, although the antibiotic resistance (AMR) and virulence profiles had changed. The most recent isolates had lost *blaTEM-1* and *erm(B)* but firmly retained *mph(A)*. Our work demonstrates the importance of collaboration between genomic and population epidemiologists, to gain insight into emerging threats to public health and to predict and prevent gastrointestinal disease.

A252

Synthetic genomics: Deep learning building blocks for the virtual genome

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Abstract

Virtual synthetic human patient genome data can now be generated and used in cases where privacy could be compromised (for example Synthea^{TM}). It is therefore timely to consider synthetic genome generation for microbes. The uses for synthetic microbial sequences include the provision of a more balanced dataset for various types of statistical analysis such as genes present/absent across specific phylogenetic clades. In addition, synthetic genomics could herald the 'invention' of novel yet similar gene clusters of a particular interesting type such as for antibiotic biosynthesis or enzymes involved in bioremediation. Could they also inform us about evolution and/or protein structure?

A deep learning neural network model for the production of coronavirus spike protein sequences was previously created with a view to examining spike protein sequences that could possibly arise in variants to aid with future-proofing vaccines. Within the current study, a model capable of generating full length DNA genome sequences of coronavirus is described. The model is able to generate virtual synthetic genomes which are either of full 100% accuracy, have single nucleotide polymorphism (SNP) changes or larger scale deletion/rearrangements. Where found, deletions occurred at short repeats within the sequence. Although greater accuracy might be achievable by increasing the network complexity, a simpler model can generate many thousands of base pairs in a short time frame and with less compute requirement. Synthetic genome sequences can subsequently be filtered using standard bioinformatic techniques to retain those with SNP or small-scale changes. Future perspectives are discussed.

A253

Hot or Not: The Evolutionary and Ecological Consequences of Having a Mutational Hotspot or Not

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Abstract

Mutational hotspots have been described as key sources of evolutionary change. However, what are the consequences of having these hotspots?

Here we take two Pseudomonas fluorescens strains, one with a mutational hotspot (SBW25) and one without (Pf0-1) and compare their relative fitness, mutation spectra and how well they grow in different nutrient environments. We also attempt to investigate the level of influence the mutational hotspot has by reciprocally inserting and removing it into each strain with varying degrees of intact genetic background.

In SBW25 the hotspot determines the route of evolution with almost 100% predictability, down to the level of a single nucleotide. In Pf0-1 we see a much more diverse mutational spectrum, with biases towards mutations in certain loci dependent on nutrient environment. When we introduce the hotspot site into Pf0-1 it gains a mutational profile very similar to ancestral SBW25. Similarly when we introduce the non-hotspot site into SBW25 it gains mutations like those in ancestral Pf0-1. When the entire hotpot-locus is removed from SBW25, we see the same outcome: a greater variety of mutations and mutation biases in specific nutrient environments. Of the mutants tested, it appears that the SBW25 hotspot mutant is not the fittest, but merely the one that can arise fastest due to its accessibility.

The "funnelling" nature of mutational hotspots down one evolutionary route can lead to other viable mutations within a population remaining undiscovered and may limit their ability to reach higher fitness peaks, as well as reduce sensitivity to environmental conditions.

A254

Screening and phenotypic characterisation of *Salmonella* Typhi colonies that have undergone genomic rearrangement

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Abstract

Like several other medically important bacterial pathogens*,* *Salmonella enterica* serovar Typhi (*S*. Typhi), the causal agent of typhoid fever, has been shown to undergo structural chromosome rearrangements. In *S.* Typhi, this is known to occur within the human host and also under certain environmental conditions. Typically, homologous recombination takes place around the seven ribosomal RNA (*rrn*) operons and leads to inversions and translocations of large genome fragments. Though this phenomenon

has been known for several decades, the evolutionary advantage is not well understood.

In this project we investigated the metabolic impact of genome rearrangement in *S*. Typhi. Long-range PCR targeting the *rrn* operons was performed as a screening method to identify colonies with a different arrangement to the parent strain BRD948 GS2.66. Colonies with potential new arrangements were cultured, had DNA extracted using RevoluGen PuriSpin Fire Monkey Kit and were long-read sequenced on the Oxford Nanopore MinION. The metabolic capacity of carbon source utilisation for several different arrangements was characterised using Biolog Phenotype MicroArrays.

Long-range PCR enables us to screen tens of colonies at a time to prioritise for sequencing those which did not produce the same bands as the parent strain. For each arrangement tested, its ability to utilise 90-180 carbon sources was assayed and compared against previously generated gene expression data.

Understanding the metabolic impact of genome rearrangements in *S*. Typhi provides insights that help to explain why such large-scale changes are tolerated and may even promote survival in humans and/or under particular environmental conditions.

A255

Comparison of acid stress responses and effects of indole across laboratory, pathogenic, and commensal E. coli

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Abstract

Escherichia coli (*E. coli*) has multiple acid resistance systems which protect the cells against low pH. One of these systems is AR2, which requires the presence of amino acid glutamate in the growth media. AR2 can be activated by exposing exponential phase cells to a pH of 5.5 for 30-60 mins. Cells respond to this induction by showing increased levels of survival upon exposure to pH levels of 2.5 and below. In our study we are comparing the survival of different *E. coli* species such as commonly used K-12 lab strains- BW25113 and MG1655, the pathogenic strain O157:H7 and a probiotic strain Nissle 1917, when exposed to extreme acid stress at pH 2.5 with and without their AR2 system being induced at different growth stages such as exponential, late-exponential, and stationary phase. Furthermore, we are examining how the bacterial signalling molecule indole can inhibit the induction of AR2 system by either directly or indirectly binding to EvgAS, one of the two component systems involved in induction of AR2 system. We assess the survival of different strains exposed to pH 2.5 with and without being induced and in the presence and absence of indole using plating assays. We further explore the mechanism of AR2 induction as well as indole inhibition in above strains using high-throughput techniques such as TraDIS (transposon directed insertion-site sequencing), the results of which will be presented in the poster.

A256

Enterobacteriaceae and *Bacteroidaceae* provide resistance to travel-associated intestinal colonisation by multi-drug resistant Escherichia coli

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Abstract

Previous studies have shown high acquisition risks of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) among international travellers visiting antimicrobial resistance (AMR) hotspots. Although antibiotic use and travellers' diarrhoea have shown to influence the ESBL-E acquisition risk, it remains largely unknown whether successful colonization of ESBL-E during travel is associated with the composition, functional capacity and resilience of the traveller's microbiome. The microbiome of pre- and post-travel faecal samples from 179 international travellers visiting Africa or Asia was profiled using whole metagenome shotgun sequencing. A metagenomics species concept approach was used to determine the microbial composition, population diversity and functional capacity before travel and how it is altered longitudinally.

Neither the microbial richness (Chao1), diversity (effective Shannon) and community structure (Bray-Curtis dissimilarity) in pre-travel samples, nor the longitudinal change of these metrics during travel, was predictive for ESBL-E acquisition. A zero-inflated two-step beta-regression model was used to determine how the longitudinal change in both prevalence and abundance of each taxon was related to ESBL-acquisition. There were detected increases to the both the prevalence and abundance of *Citrobacter freundii* and two members of the genus *Bacteroides*, in association with remaining uncolonised by ESBL-E.

These results highlight the potential of these individual microbes as a microbial consortium to prevent the acquisition of ESBL-E. The ability to alter a person's colonisation resistance to a bacterium could be key to intervention strategies that aim to minimise the spread of MDR bacteria.

A257

Phylogenetic analysis of the change in *Escherichia coli* population during international travel

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Abstract

Strains of *E. coli* can be separated by their divergent genetic profiles into distinct phylogenetic groups (phylogroups), which often vary by geography or pathogenicity. Human commensal *E. coli* belong predominantly to phylogroups A and B1 and usually have lower levels of virulence compared to phylogroups B2 and D. However, the commensal population of *E. coli* can harbour antimicrobial resistance genes and transfer these to pathogenic strains, so it is vital to monitor the commensal *E. coli* population. Pre- and post-travel faecal samples from 179 international travellers visiting Africa and Asia were shotgun sequenced and the data was assembled into metagenome assembled genomes (MAGs). 212 *E. coli* MAGs were selected and compared to 420 reference *E. coli* sequences of 21 different sequence types, to identify commensal pre-travel *E. coli* strains and which strains were acquired internationally.

The population of *E. coli* strains is drastically different between pre-travel and post-travel samples. The commensal population of 90 *E. coli* MAGs was predominated by phylogroup B2 (33/90), followed by phylogroups A (24/90) and D (14/90). Whereas the post-travel acquired *E. coli* MAGs belonged primarily to phylogroups A (60/122) and B1 (29/122). No traveller acquired *E. coli* phylogroup B2 during travel. These results highlight that the commensal *E. coli* population is more dominated by phylogroup B2 than commonly thought, and how international travel can drastically alter the phylogroups that are present. It is vital to monitor how the commensal population of *E. coli* may shift in its phylogroups, virulence and level of antimicrobial resistance.

A258

Conditional evolution of biocide tolerance in nosocomial pathogens

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Abstract

Biocides are widely used to control and prevent healthcare-associated infections. The emergence of bacterial resistance to biocides and cross-resistance to antibiotics is a major issue of concern. Biocides often must eradicate bacteria in biofilms which are highly tolerant and pose a challenge in infection control.

Understanding how major hospital pathogens respond to different biocidal agents when grown as biofilms is key to improve infection prevention and control. A biofilm evolution model was used to study the evolutionary changes that occur in *Staphylococcus aureus* and *Enterococcus faecalis* when exposed to biocides. Biofilm (on steel beads) and planktonic lineages were exposed to sub-lethal concentrations of Chlorhexidine digluconate (CHG) and Octenidine dihydrochloride (OCT) in parallel for up to 250 generations.

E. faecalis biofilms were able to survive escalating CHG concentrations (up to 32 µg/ml); biofilm productivity was maintained until growth stopped. Planktonic lineages were able to survive at higher concentrations of CHG (up to 128 µg/ml). When exposed to OCT, biofilm lineages were unable to adapt beyond the MIC, but planktonic lineages were able to survive higher OCT concentrations although with poor growth. Similar results were observed for *S. aureus*.

Phenotypic characterization to assess biofilm-forming capacity did not show an increase in the biofilm biomass in evolved isolates, antibiotic MICs were determined to identify any cross resistance and genome sequencing was used to identify the genetic basis for adaptation.

Biofilms do not readily develop resistance to OCT and CHG in two major nosocomial pathogens although planktonic cultures demonstrate higher tolerance can evolve.

A259

Evaluating the interconnection of the microbiome and resistome of farm livestock and their environment

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Abstract

Comprehensive characterisation of livestock gut microbiomes is necessary for understanding antibiotic resistance in farming environments. Within these microbiomes opportunistic pathogens, such as *Escherichia coli* and *Salmonella enterica*, can thrive and gain a large repertoire of antimicrobial resistance. Here, we conducted large-scale sampling across 10 poultry farms and slaughterhouses spanning different geographic regions in China, to investigate microbiomes and resistomes as well as *E. coli* and *S. enterica* isolates in different microbial communities: broiler, farm environment, carcasses, and abattoir environment. Samples were analysed using antibiotic susceptibility testing, metagenome and whole genome sequencing, machine learning (ML), genome-scale modelling, Bayesian and maximum likelihood phylogeny, and 3D structure mutational analysis. Antibiotic resistance genes (ARGs) were found to be widespread within each community regardless of the usage of antibiotics. In addition, several clinically relevant ARGs and associated mobile genetic elements were shared across different communities. Aggregating metagenome and isolate-based methods together with sensor data we further assessed the temporal and geographical effects, as well as the temperature and humidity on microbial populations and resistomes. Comparing *E. coli* and *S. enterica* isolates we found differentiations in both the phylogenetic evolution and features underlying antibiotic resistance however genome-scale models powered by machine learning revealed a large overlap of metabolic pathways associated with antibiotic resistance. Our results demonstrate the importance of adopting a multi-domain and multi-scale approach when studying microbial communities and AMR in complex, interconnected environments.

A260

Understanding the Contribution of the Human Virome in Pregnancy, Early Life, Aging, and Cognitive Impairment

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Abstract

The human body is host to extensive microbial communities known collectively as the microbiome. The human virome comprises all the viruses that coexist in a healthy human, with each person carrying an estimated $>10^{12}$ viral particles with significant heterogeneity in composition. Of these collections the gastrointestinal virome is the largest and has significant implications in health and disease. Recent studies have explored the viral diversity from a number of body sites using metagenomic sequencing. Most of the viruses within these samples are uncharacterised or uncultivated, highlighting the vast amount of viral 'dark matter' that exists. The gut virome is abundant with bacteriophages that have been shown to shape bacterial populations, and the viral component of the microbiome may be a missing link between bacteria and the host immune system. Development of a healthy gut is key for mitigating many diseases, and the virome is likely an important player in key stages across life, such as pregnancy, early life and old age. Importantly, how changes in the microbiome and virome relate to mechanistic changes in the body remains largely unknown. We at the Gut Microbes and Health programme at the Quadram Institute are investigating these aspects of the microbiome through two longitudinal human studies; one that aims to understand the mechanisms behind healthy microbiome establishment in pregnancy and early life (PEARL) and the other to understand diseases associated with aging such as cognitive impairment (MOTION).

A261

Diversity of the *Enterobacter cloacae* complex in the faecal microbiota of preterm infants

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Abstract

Preterm infants are at high risk of nosocomial infection. The *Enterobacter cloacae* complex (ECC) has been reported in nosocomial outbreaks in neonatal intensive care units and is associated with a high mortality rate, but few studies have characterized the ECC in this cohort. Members of the ECC carry numerous antimicrobial resistance (AMR) genes, and contribute to the resistome. The aim of this study was to understand the genomic diversity of the ECC and identify species of the complex found in the preterm infant gut microbiota. Using a previously published dataset of 262 metagenomic samples, we performed taxonomic classification (Kraken2) and abundance re-estimation (Bracken). Using metabat2, metagenome-assembled genomes (MAGs) were recovered from 51 samples with relative abundance of ECC >1 %. Nine high-quality ECC MAGs (completeness ^{3}90 %, contamination £5 %) were obtained with average nucleotide values >95 % to 23 ECC reference genomes. All the MAGs were classified into three clades comprising *Enterobacter hormaechei* subsp. *steigerwaltii*, *Enterobacter hormaechei* subsp. *oharae*, and *Enterobacter hormaechei* subsp. *hoffmanii*. Pangenome analysis was performed on the MAGs using Anvi'o. Multi-locus sequence typing, and AMR gene, prophage region and CRISPR-Cas profiling were performed to characterize the MAGs. b-Lactamase was founded in all nine MAGs. Only three MAGs represented known sequence types: ST133, ST104, and ST68. Phage regions were identified in all MAGs. Five out of nine MAGs had CRISPR arrays. In conjunction with our analyses of over 2000 ECC genomes, this work increases our understanding of the genomic diversity of ECC members contributing to infection.

A262

AN INVESTIGATION INTO THE EFFECTS OF FREEZING ON THE MICROBIOME OF HUMAN CADAVERS

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Abstract

Studies on the human microbiome for forensic applications, particularly regarding estimation of postmortem intervals, are on the rise. Many of these studies often involve cadavers which have been previously frozen and are then thawed prior to commencing research. While this cycle of freezing and thawing cadavers is common practice, its effect on the process of decomposition and the development of the (thanato)microbiome has not been extensively researched and documented. This study aims to understand how freezing cadavers can affect the microbiome as significant effects from freezing could skew experimental data and the resulting interpretation.

To investigate the effects of freezing on the microbiome of cadavers, we collected swab samples from five anatomical locations of nine cadavers (oral, rectum, hand, foot, and neck). Cadavers were swabbed on arrival to the human taphonomy facility, and again after freezing at –20 °C and thawing. Samples are currently being processed by metabarcoding of the 16S rRNA region of the DNA extracts; however, results will be ready in time for the conference.

This study will be essential for establishing and/or reviewing standard operating procedures on sampling human taphonomy facilities worldwide and will provide insight into the validity of using previously frozen cadavers in microbiome studies. The results may also provide useful information on microbial composition and abundance for forensic cases involving frozen bodies.

A263

MICROBIAL SIGNATURES IN THE ORAL MICROBIOME AS A POTENTIAL TOOL IN FORENSIC HUMAN IDENTIFICATION

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Abstract

The oral cavity has been classified as an area with one of the largest and diverse microbial communities in the human body. The constituents of this community have been shown to vary among individuals as they are influenced by various factors. This variation in individuals presents new implications for forensic investigations regarding human identification as the oral microbiome can potentially be used as a new type of evidence.

However, significant gaps in knowledge concerning how factors such as age, sex, geographical provenience, etc. affect the oral microbiome is still largely unknown. Thus, the aim of this study is to add to existing knowledge by examination of the oral microbiome of individuals and their lifestyles.

For this study, 50 individuals residing within Italy donated oral swab samples and provided information regarding their diet, lifestyle, health status, antibiotic use and other personal data. Skin swabs from 11 of the 50 individuals were also analyzed and compared to the oral swabs from the same donors. All analyses were done through metabarcoding of the 16S rRNA region of DNA extracted from the samples.

The results showed the presence of a core microbiome within all the samples. It also indicated microbial signatures associated with a specific individual or certain grouping conditions or habits. This has significant implications for forensic investigations for which the ability to differentiate is essential.

This research outlines the potential use of oral microbiome signatures in forensic human identification and in providing investigative information regarding the lifestyle of the host donor.

A264

Mutant selective advantage and pharmokinetics drive the low incidence of Nitrofurantoin resistance in uropathogenic Escherichia coli.

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Abstract

Nitrofurantoin was first used to treat urinary tract infections since the 1950s. Recently, it has been re-introduced as the first-choice antibiotic to treat uncomplicated acute UTI in the UK. Highly effective against common uropathogens, such as *Escherichia coli*, its use is accompanied by a low incidence (<10%) of antimicrobial resistance. Nitrofurantoin mode of action requires the reduction by nitroreductases, generating electrophilic intermediates that inhibit protein and nucleic acid synthesis. Resistance to Nitrofurantoin is predominantly via the acquisition of loss-of-function, step-wise mutations in the nitroreductase genes *nfsA* and *nfsB*.

Prophylactic use of Nitrofuranotin in the clinical trial AnTIC, showed that prophylaxis reduces the incidence of UTI by one per annum. Analysis of longitudinal uropathogenic *E. coli* isolates from AnTIC, identified 2 case examples of Nitrofurantoin resistance evolution. Growth rate analysis identified a 2-10% slower doubling time for Nitrofurantoin resistant strains. These data suggests there is no fitness advantage of evolved strains over their sensitive predecessor. Genetic manipulation of *E. coli* to mimic Nitrofurantoin resistance evolution, confirmed no fitness advantage was gained. Further analysis argued that a first-step mutant gained a selective advantage, rather than a fitness advantage, at sub-MIC (4-8 mg/L) Nitrofurantoin concentrations.

Correlation of our data to Nitrofurantoin pharmokinetics argues that: the low incidence of resistance is via urine-based Nitrofurantoin concentrations that inhibit the growth of *E. coli* strains carrying the key first-step loss-of-function mutation. We argue that providing greater insight into the correlation between antibiotic behaviour in patients and AMR can aid clinical decisions on treatment strategies for UTI.

A265

The Multiple Antibiotic Resistance Activator (MarA) induces persister cell formation by regulating metabolic genes

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Abstract

Multiple antibiotic resistance in bacteria can be driven by the transcriptional regulators in the AraC-XylS family. The *Escherichia coli* '*mar*' regulon is considered a paradigm for such systems. The *mar* locus consists of 3 genes; *marR*, *marA*, and *marB*. A transcriptional activator encoded by *marA* enhances drug resistance by binding to "marbox" sequences at target promoters. Previously, we identified 33 MarA targets by ChIP-Seq analysis in a pathogen, Enterotoxigenic *E. coli* (ETEC) and reported two mechanisms of resistance to quinolones and tertacyclines. Further analysis of MarA targets has revealed that some metabolic genes that are involved in nucleotide and amino acid biosynthesis are regulated by MarA. Here, we show that MarA regulation of these genes inhibits growth and thereby induces persister cell formation.

A266

Temporal GWAS of *Shigella* identifies novel genetic drivers of long-term success

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Abstract

Bacteria of the genus *Shigella* are a major contributor to the global diarrhoea burden causing >200,000 deaths per annum globally. Increasing antibiotic resistance in *Shigella* and the lack of a licenced vaccine has led WHO to recognise *Shigella* as a priority organism for the development of new antibiotics. Understanding what drives the long-term persistence and success of this pathogen is critical for ongoing global management of shigellosis and has relevance for other enteric bacteria.

To identify key genetic drivers of *S. flexneri* evolution over the past 100 years, the unique and significant potential of historical bacterial genomes was utilised. The historical Murray collection, comprising several hundred pre-antibiotic era (1917 – 1954) *Enterobacteriaceae,* was used alongside comparatively modern (1950s – 2018) isolates to conduct genome-wide association studies. We identified 94 SNPs and kmers within 48 genes significantly positively associated with time as a continuous variable. These included genes encoding T3SS proteins, proteins involved in intracellular competition, and multidrug resistance proteins which have intuitively beneficial roles for *Shigella* as pathogens. However, 34% of identified hits related to genes of as yet unknown function. Subsequent Alphafold modelling of one such protein has identified one of these hits as a putative novel adhesin.

Thus, our temporal GWAS approach, has identified known and novel genetic factors that are enriching in *Shigella* populations over time. These genetic determinants may thus be key factors in the long-term success of *Shigella* as well as offer potential targets for pathogen management.

A267

Mobile genetic elements and carbapenem-resistance in Gram-negative pathogens – lessons from the DETECTIVE project

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Abstract

China is one of the top consumers of antibiotics globally. Since 2019, the DETECTIVE project has investigated *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli* from intensive care unit (ICU) environments, patients and staff in Hangzhou, Chengdu and Guangzhou, China. Whole-genome sequences were obtained for over 1500 isolates, over 100 of which were long-read sequenced. High-resolution annotation, comparative analyses and laboratory experiments were employed to characterise plasmids and translocatable genetic elements associated with the acquisition, maintenance and transmission of ESBL and carbapenem resistance genes (ECRGs). A database was created for typing the understudied plasmids of *Acinetobacter in-silico*.

We used nucleotide 'signatures' generated by molecular events that shape evolution to identify and trace distinct lineages amongst a diverse array of ECRG-bearing plasmids. This approach allowed us to detect plasmid transfer in ICU environments and identify successful lineages found in different *E. coli* and *K. pneumoniae* sequence types from patients, staff and ICU environments, as well as in agricultural and human commensal isolates from within and outside China. Particular plasmid lineages were also implicated in the chromosomal acquisition of IS*Ecp1*-$bla_{\text{CTX-M}}$ transposition units by some *E. coli*. Two plasmid families associated with $bla_{\text{NDM-1}}$ in *Acinetobacter* were characterised for the first time, revealing that they are mercurial mosaics that apparently diversify through XerC/D-mediated recombination.

The evolution of ECRG-bearing plasmids highlights the emerging importance of cointegration events that fuse unrelated plasmids and might impact their host ranges, transmission and stability. Lineage-specific surveillance will be crucial for tracking the global dissemination and ongoing evolution of plasmids.

A268

The effect of antimicrobial resistance-causing mutations on the evolution of further resistance.

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Abstract

Mutations can allow bacteria to acquire resistance to antibiotics and at the same time, they may affect cell fitness and sensitivity towards other antibiotics. The genetic background of a bacteria may also influence the effects of such mutations. For example, the same resistance-conferring mutation in two different genetic backgrounds can provide different levels of resistance and fitness effects. Thus, understanding the nature of these mutations with respect to genetic background is important as it could help us develop methods to slow down or reverse the rapid evolution of antimicrobial resistance in bacterial populations. The main aim of this project is to examine whether mutations that confer resistance to one antibiotic affect the evolvability to a second antibiotic in *E. coli* K-12. To measure evolvability, we quantify the mutation rate of resistant mutants against a second antibiotic and compare them to the wild type. To measure mutation rate we use fluctuation assays. We also perform mathematical simulations of the assay to optimise conditions and explore our ability to detect differences in evolvability experimentally. Finally, we aim to assess whether the order in which resistance to two different antibiotics is acquired affects the final level of resistance and fitness. The results from this project will provide insight into how genetic interactions may affect resistance, fitness and evolvability. This could be important for designing treatment strategies that minimise the risk of resistance evolution.

A270

Genomic diversity of Klebsiella species in the human gut

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Abstract

Antibiotic-resistant *Klebsiella pneumoniae* has become a growing cause of nosocomial and community infections, posing a significant public health problem. There is currently a lack of information on the diversity and antibiotic resistance mechanisms in *Klebsiella* strains that colonise the human gut. The current study focuses on human stools samples which were collected in a randomised clinical trial on the prevention of traveller's diarrhoea by the administration of prophylactic rifaximin in British soldiers deployed to Kenya. Faecal samples were collected at the start of deployment and after six weeks in Kenya and *Klebsiella* was isolated on SCAI medium after overnight enrichment in Luria Bertani broth with ampicillin. *Klebsiella* was detected in 81 (33.5%) of 242 stool samples. The presence of *Klebsiella* was not different among the placebo and rifaximin treatment groups (X^2: 0.6523, $P>0.05$). Whole-genome sequence analysis of 76 strains revealed a significant variation in *Klebsiella* species across different individuals. Multiple *Klebsiella* species, including *Klebsiella pneumoniae* (47%), *Klebsiella variicola* (32.0%), *Klebsiella michiganensis* (7.8%), *Klebsiella grimontii* (2.6%), *K. pasteurii* (1.3%), *K. quasipneumoniae* (1.3%) and *K. oxytoca* (1.3%) were identified. In one individual, the same clone of *K. variicola* was found at the start and end of study period, while three participants were found to have identical clones of *K. pneumoniae.* Notably, identical *K. variicola* strains were present in multiple individuals, which strongly suggested acquisition from a single shared point-source. Our data show that *Klebsiella* colonisation of the human gut is mostly transient. Resistance in *K. variicola* may deserve further attention as a potential threat to public health.

A272

Adding metabolic function to the genome of Salmonella Enteritidis responsible for bloodstream infection

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Abstract

Invasive non-typhoidal Salmonella disease (iNTS) is a bloodstream infection that predominantly occurs in sub-Saharan Africa. Salmonella enterica serovar Enteritidis is responsible for one third of iNTS infections. Clades of S. Enteritidis have been identified that are associated with either gastroenteritis (Global Epidemic clade) or the epidemic of bloodstream infection (African clades). However, the genetic basis of the distinctive metabolic properties of S. Enteritidis remains unknown. This project aimed to investigate clade-specific metabolic differences using a combination of physiological characterisation and genomic analysis.

During my Harry Smith Vacation studentship, I compiled and curated the results of a range of carbon utilisation experiments that involved 138 S. Enteritidis isolates, 122 of which had sequenced and annotated genomes. A phylogenetic tree was constructed and contextualised with source data and carbon-utilisation profiles.

I identified a clade-specific difference that distinguished the Global Epidemic clade from the African clade, involving Galactitol utilisation. A gene comparison of the galactitol utilisation pathway between S. Enteritidis and S. Typhimurium showed a difference in the number and organisation of the gat operon genes. Multiple sequence alignments were used to identify a SNP that pseudogenises the gatC gene in the African clade isolates, compared to the Global Epidemic clade. As gatC encodes a galactitol transporter, I hypothesise that this SNP is responsible for the observed galactitol utilisation defect of the African clade isolates. This differential galactitol utilisation may reflect the adaptation of African clades of S. Enteritidis from a gastrointestinal to a systemic lifestyle.

A273

Unrecognized contribution of the novel species *Escherichia marmotae* to zoonotic transmission and antimicrobial resistance

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Abstract

Reliable detection methods and accurate information are essential to uncover emerging pathogens and antimicrobial resistance (AMR) transmission in the context of the One Health perspective. MALDI-ToF MS is routinely performed for bacterial species confirmation in the German national monitoring program of zoonotic and commensal *Escherichia coli*. Twenty-four presumptive *E. coli* achieved MALDI scores <2.3, which indicated unreliable species identification. Whole-genome sequencing identified isolates as *E. marmotae*, a species recently identified in China. *E. marmotae* were isolated from vegetable, meat products and feces of wild boars in Germany, of which nine isolates exhibited phenotypic resistance to colistin, a last-resort antimicrobial for the treatment of infections with multidrug-resistant bacteria in humans.

Phenotypic and biochemical characterization revealed greater similarity of the isolates to the *E. coli* than to the *E. marmotae* reference strain. In light of the One Health perspective, comparisons to 32 publicly available *E. marmotae* genomes, obtained from human infections, livestock and the environment, uncovered substantial differences in the phylogenetic relationship and represented over 20 MLST and more than 27 different cgMLST. Importantly, *E. marmotae* encoded a variable arsenal of virulence-associated genes. Colistin resistance was mediated by a yet unknown non-transferrable resistance mechanism.

E. marmotae is adapted to different biological niches likely to hold zoonotic and human pathogenic potential highlighting the need for improved methods to distinguish the novel *Escherichia* species from *E. coli* in existing monitoring programs. Notably, all isolates belonged to the cryptic clade V and carried a H56 flagellar antigen, possible indicators for *E. marmotae* identification during routine diagnostics.

A274

Compartive benchmarking of metagenomic phage identification tools.

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Abstract

As the relevance of bacteriophages in shaping diversity in microbial ecosystems is becoming increasingly clear, the prediction of phage sequences in metagenomic datasets has become a topic of considerable interest, which has led to the development of many novel bioinformatic tools. A comparative analysis of these tools was carried out to inform the usage of them in research workflows. We benchmarked eleven state-of-the-art phage identification tools. Artificial contigs generated from complete RefSeq genomes representing phages, plasmids, and chromosomes, and a previously sequenced mock community containing four phage strains were used to evaluate the precision, recall and F1-scores of the tools. In addition, a set of previously simulated viromes was used to assess diversity bias in each tool's output, which is essential to ecological studies. VirSorter2 achieved the highest F1 score (0.92) in the RefSeq artificial contigs dataset, although several other benchmarked tools also performed well. Kraken2 had the highest F1 score (0.86) in the mock community benchmark, mainly due to its high precision (0.96). Generally, k-mer based tools performed better than reference similarity tools and hybrid methods. When analysing the diversity of the genomes that each tool predicted from a virome set, most tools produced a viral genome set that had similar alpha and beta diversity patterns to the original population. The notable exception was Seeker, whose metrics differed significantly from the diversity of the underlying data.

A275

Understanding the spread of multi-drug resistant megaplasmids in *Pseudomonas aeruginosa*

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Abstract

Plasmid-mediated multi-drug resistance in bacteria is a major challenge and threat to global health. Owing to their large size, vast geographical distribution and potential to transfer multiple resistance genes at once, *Pseudomonas* 'pBT-family' megaplasmids pose a potential epidemiological threat as vectors of anti-microbial resistance (AMR). pBT-family megaplasmids have been found in diverse species, including *P. koreensis*, *P. shirazica* and *P. aeruginosa*, where they have been found to confer high levels of resistance to key frontline antibiotics. However, the risks of horizontal transfer of AMR due to pBT-family megaplasmids is unclear due to a lack of experimental data investigating transmission and maintenance of these megaplasmids in diverse strains. Here, we tested 81 diverse *P. aeruginosa* strains for their ability to acquire and maintain the pBT-family megaplasmid pOZ176. This plasmid could transfer to all but 5 (93%) of the *P. aeruginosa* strains tested, and plasmid-bearing strains did not lose pOZ176 when cultivated for ~70 generations without any antibiotic selection, suggesting that plasmid conferred benefits tend to surpass plasmid-imposed fitness costs across diverse *P. aeruginosa* strains. Although pOZ176 could transfer into diverse recipients, its phenotypic effects were divergent, particularly in terms of its effects on exopolysaccharide production, which varied from positive to negative across recipients. Phylogenetic analysis of these strains has further added to our understanding on acquisition of these plasmids by various strains. Identifying the lineages of *P. aeruginosa* that are more susceptible to megaplasmid- mediated AMR would help us in identifying the strains with potential epidemiological threat.

A276

Against all odds: codon-usage-driven maintenance of numerous frameshifts in the mitochondrial genomes of four *Perkinsus* species

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Abstract

The mitochondrial genomes of *Myzozoa* (apicomplexans, dinoflagellates and chrompodellids) are uncommonly reduced in coding capacity, presenting only genes for COX1, COX3 and COB. Their rRNAs are highly fragmented, and their organization is affected by pervasive recombination. Partial gene sequences of myzozoan *Perkinsus cox1* and *cob* have been previously reported, revealing independent acquisition of the unusual feature of multiple encoded frameshifts. Here, we sequenced and assembled the complete mitochondrial genomes of four *Perkinsus* species. Our genomes show typical myzozoan reduced coding capacity, including our identification of a divergent *cox3*, and genomes prone to rapid rearrangement likely due to rapid recombination. Across the four species there are 75 highly conserved frameshifts with the majority resulting in glycine residues across all three genes but also proline residues exclusive to *cox1*, and asparagine residues exclusive to *cob*. The presence of numerous and highly conserved frameshifts implies a positive function of these features and a mechanism for efficiently decoding the frameshifts. Codon-usage analyses around the frameshift sites provided the basis for a simple mechanism that can effectively explain all predicted frameshifts. At each site the coding sequences encounter an unused codon necessitating a shift in the translation frame by one or two nucleotides until the next used codon is reached. This process might be assisted by the mRNA structure and modelling reveals a conserved ~30 nt motif at most frameshift sites. *Perkinsus* demonstrates unusual features and remarkable trajectories of endosymbiont genome evolution, most notably the pervasive integration of frameshift translation into genome expression.

A277

Evidence for local and international spread of *Mycobacterium avium* subspecies *paratuberculosis* through whole genome sequencing of isolates from the island of Ireland

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the agent of Johne's Disease (JD) in cattle, a chronic enteritis that is a threat to animal health and productivity. Whole genome sequencing (WGS) is being increasingly used to understand MAP infection and here we present the first comprehensive WGS study of MAP on the island of Ireland, encompassing 197 isolates.

WGS revealed MAP across the island is genetically diverse, forming 8 clades separated by ~200 SNPs. Conversely, the classical typing method of mycobacterial interspersed random unit – variable number tandem repeats (MIRU-VNTR) suggested low genetic diversity of Irish isolates, with 95.4% of isolates corresponding to two MIRU-VNTR types, highlighting the utility and superior resolution of WGS. We found evidence of MAP transmission between Irish herds, with the same strain(s) present in multiple herds. There was likewise evidence of mixed infection with different MAP strains present in herds.

Comparing our isolates to published European isolates showed multiple instances of genetic similarity. Structured coalescent analyses (MASCOT) revealed a higher amount of transitions from Europe to Ireland than the reverse, as well as a higher effective population of MAP in Europe. Our analysis also indicated that Irish and European MAP isolates share common ancestry in the last 50-100 years, which is further supported by historic cattle trading trends across the continent.

In summary, this work provides new insight into MAP genetic diversity across the island of Ireland, placing it in a global context and providing the basis for extending WGS to understand MAP transmission dynamics.

A278

Bottlenecks of selection and two-stage emergence of a zoonotic clade of *Streptococcus suis* from a background of swine-associated *Streptococcus spp*. in SE Asia

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Abstract

Streptococcus suis commonly causes disease in pigs worldwide and is increasingly implicated in zoonotic disease in South-East Asia. We investigated the evolutionary history of *S. suis* with a combined population genetic-phylogenetics approach using whole genome sequence data from healthy and diseased pigs sampled in Northern Thailand in addition to publicly available *S. suis* genomes from human infection in SE Asia. Genomes from healthy pig isolates were significantly larger than disease isolates with a greater number of open reading frames. Genealogical reconstruction of isolates from clinical infection, healthy and diseased farmed pigs revealed a complex genetic structure. Broadly, the majority of healthy, diseased pigs and clinical infection isolates clustered together suggesting recent clonal decent. Our isolates from healthy pigs demonstrate a large pool of genetic diversity in pigs from which disease isolates have descended, followed by a second bottleneck which has given rise to a clonal population of isolates that can cause disease in humans. Using a genome-wide association study we compared healthy and disease pig isolates to identify disease-associated genetic elements. Among the genes we identified with the strongest association with disease were several genes involved in DNA repair, transcription, modification, and replication. Antimicrobial resistance genes were also found to be associated with pig disease, including tetracycline, erythromycin, beta-lactam and multi-drug efflux proteins which are all commonly used in agricultural practices in SE Asia.

A279

Modulation of Gene Expression in *Streptococcus pneumoniae* by Methylation of the Type I Restriction Modification System SpnIII

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Abstract

Methylation by the type I restriction-modification system (RMS) SpnIII in *Streptococcus pneumoniae* is hypothesised to regulate gene expression via epigenetic changes (Manos). The phase variable SpnIII generates six host specificity determinants (*hsdS*) alleles through site-specific recombination, each of these alleles corresponds with different methylation pattern (DeSteCroix).

In addition to known roles of the RMS in restricting phage infection and transformation, we have now tested the impact on gene expression. RNAseq was used to analyse *S. pneumoniae* strains expressing a single *spnIII* allele (*spnIII*A, *spnIII*B or *spnIII*E) to determine differences in gene expression profiles. The data have identified few genes which show differential expression and have a methylation site mapping to their predicted promoter region. Seven synthetic promoters with the wild type and altered methylation target site were cloned in front of a luciferase gene in strains expressing a single *spnIII* alleles. For the analysed constructs, data indicate that the methylated promoters show a three- to twenty-fold higher activity compared to non-methylated promoters for those genes upregulated as per RNAseq data whereas downregulated genes have higher expression from the non-methylated as opposed to the methylated promoter. Results obtained were further confirmed by qPCR analysis. Preliminary data using drugs targeting DNA topoisomerases indicate that the mechanism by which methylation impacts gene expression could be related to DNA topology. An RMS dependent modulation of gene expression is demonstrated for the first-time here, proposing an alternative mechanism of epigenetic regulation.

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A280

TyphiNET: An online dashboard for global genomic surveillance of *Salmonella* Typhi

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Abstract

Typhoid fever is a faeco-orally transmitted systemic infection caused by *Salmonella* Typhi (*S.* Typhi). Each year >10 million cases occur worldwide of which >100,000 are associated with mortalities making it a public health threat in many low- to middle-income countries with limited hygiene and sanitation infrastructure. Whole genome sequencing (WGS) and core-genome phylogenetics have become the standard for typhoid molecular epidemiology in both research and public health settings, providing insights into population structure, transmission dynamics, antimicrobial resistance (AMR) emergence and dissemination, as well as outbreak investigation and monitoring of intervention strategies. Despite this, WGS data are not united and summarised in a single surveillance platform for maximum public health benefit. Here we present TyphiNET; an automated online surveillance dashboard available at http://typhi.net developed as a JavaScript application. TyphiNET imports public data from Typhi Pathogenwatch (available at: https://pathogen.watch/) producing interactive visual summaries of public health utility including global and country level overviews of population structure and AMR over time. TyphiNET captures previously reported changes in the *S.* Typhi population structure including both the emergence of extensively drug-resistant (XDR) typhoid in Pakistan, and mutations associated with azithromycin resistance in South Asia. Data and visualisations can be filtered to report data from different sampling frames, time spans and geographic locations, and can be downloaded for use in presentations and reports. Subsequently, TyphiNET provides an up-to-date overview of the global *S.* Typhi population structure to better capture the emergence and spread of AMR variants, changes in population structure, and impacts of intervention strategies worldwide.

A281

ESBL-producing *E. coli* from German meat and livestock may represent a source of *mcr*-1.26 IncX4 plasmids previously observed in humans

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Abstract

Colistin remains an indispensable antimicrobial in livestock production, despite its increasing importance as last-resort antimicrobial for the treatment of infections with multidrug-resistant bacteria in humans. Since 2015, increased attention has brought to colistin resistance due to the discovery of the mobile colistin resistance (*mcr*) genes located on transmissible plasmids. Transmission of colistin-resistant bacteria and *mcr*-bearing plasmids is facilitated by advancing urbanization and anthropogenic transformation of landscapes, which forces livestock animals and humans into greater contact. Additionally, contaminated meat-based food products may serve as source for the acquisition of resistant bacteria by humans.

The *mcr*-1.26 variant was first described in 2018 in two *E. coli* isolates from hospitalized patients in Germany. *mcr*-1.26 was carried on transmissible IncX4 plasmids of 33 kb. Here, we report the presence of *mcr*-1.26 in twelve ESBL-producing and commensal *E. coli* obtained from meat and feces of poultry in Germany. *mcr*-1.26 already appeared 2014 in an isolate from turkey meat, before the first report of a *mcr*-gene. Comparable to the clinical isolates, *mcr*-1.26 was located on transmissible 33 kb IncX4 plasmids. Isolates carried additional determinants conferring resistance towards ampicillin (92%), nalidixic acid (83%), ciprofloxacin (83%), tetracycline (83%), trimethoprim (58%) and sulfamethoxazole (50%), a resistance profile similar to the clinical isolates. The veterinary isolates belonged predominantly to the sequence types ST10 and ST744. The human isolates were assigned to ST155, a common ST in poultry, and ST69, which has been described for cattle. Consequently, transmission of colistin-resistant bacteria via food or close contact to livestock is therefore possible.

A284

The role of naturally occurring plasmids in the lifestyle choices of *P. fluorescens*

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Abstract

Conjugative plasmids play an important role in bacterial evolution by transferring accessory genetic information throughout a population. It is well understood that carriage of large conjugative plasmids can lead to adaptive genetic changes to resolve genetic conflicts between the plasmid and the host. However, what is less understood is how the carriage of these plasmids can lead to phenotypic changes of their host bacteria without direct genetic manipulation. Through bioinformatic analysis we found that many conjugative plasmids encode *rsmQ*, a homolog of a global translational regulator. By using a model system, we discovered that carriage of the plasmid pQBR103 by *Pseudomonas fluorescens* caused the host to reduce its motility and increase its biofilm production. Using novel biophysical and biochemical approaches we were able to unpick the underlying molecular mechanisms resulting in these phenotypic changes. We showed that the plasmid borne protein RsmQ can directly influence the proteomic landscape of its host in the absence of compensatory genetic mutations leading to direct phenotypic changes. This work begins to uncover the complex molecular interactions between plasmids and their hosts that are not the direct result of genetic changes

A285

Effect of active prophages on the virulence of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is a key opportunistic pathogen and major cause of death in cystic fibrosis (CF) patients, partly due to establishment of robust biofilms in the lungs. The Liverpool Epidemic Strain (LES) is associated with increased pathogenicity and competitiveness in the CF lung which is thought to be influenced by several prophages present in its genome. Active prophages can destroy their host bacteria by induction of the lytic bacteriophage cycle or remain part of their hosts DNA and provide fitness advantages through the lysogenic cycle. Active phages are very common in the CF lung but little is known about the co-operative interactions between them and their bacterial hosts.

This project investigates the relationship between three active prophages of LES and the model *P. aeruginosa* host strain PAO1. In vitro culture experiments revealed that LES prophages affect bacterial host growth rate differently depending on the conditions. Challenge experiments, using the *Galleria mellonella* infection model, indicated that prophage carriage reduces bacterial virulence by between 8% and 23%. These data suggest that the LES prophages may contribute to adaptation for longer survival in vivo.

A287

Discovering genetic barriers to the spread of antibiotic resistance genes

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Abstract

INTRODUCTION

Bacteriophage facilitate a major form of horizontal gene transfer (HGT) , termed “generalised transduction”, in the human pathogen species *Staphylococcus aureus*. However, our knowledge of the barriers to HGT remains limited. This project therefore seeks to identify and characterise *S. aureus* genes with undiscovered transfer barrier functions using a novel screening methodology.

METHODS

A transposon mutant library of a clinical methicillin resistant *S. aureus* strain was screened. Each mutant was co-cultured with another strain and an 80α generalised transducing phage for 24h. The strains were differentiated by their varying antibiotic resistance gene (ARG) carriage. The rate of ARG transfer was then determined. An increased rate indicates the wildtype gene, which is knocked out in the mutant, functions to restrict transfer. Genes of interest were then characterised further.

RESULTS

Current screening shows 2.82% (23/815) of mutants exhibit increased HGT rates, demonstrating potential transfer barrier function of their respective wildtype gene. One mutant strain received a resistance plasmid at a rate 31-fold greater than the control strain. Putative analysis of the wildtype gene, *scpB*, revealed a condensin subunit function.

DISCUSSION

These results imply a dual function of *S. aureus* condensin genes, acting to partition DNA and restrict HGT. The ScpB protein may selectively affect transduced plasmid stability within the host. These preliminary results suggest that many undiscovered transfer barrier mechanisms exist within *S. aureus*, and will be extended by screening the remaining mutants. This is the first step in uncovering potential new biotechnologies and gene targets for combating the spread of ARGs.

A288

Non-canonical Staphylococcus aureus pathogenicity island repression

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Abstract

Staphylococcus aureus pathogenicity islands (SaPIs) are the prototypical members of a new family of mobile genetic elements, the phage-inducible chromosomal islands (PICIs). SaPIs are clinically important because they encode and disseminate toxin and antibiotic resistance genes. Normally, these elements reside passively integrated into the bacterial chromosome, thanks to the expression of a master repressor, called Stl. We recently solved the structure of the SaPIbov1 Stl, a canonical dimer with a structure similar to that previously reported from many phage- and other mobile genetic elements-encoded repressors. Since canonical repressors' self-cleavage break the dimer confirmation activating either the lytic cycle of the prophages or the transfer of the different MGEs, SaPIbov1 Stl is disrupted after its interaction with its congnate phage-encoded inducer proteins. The existence of multiple Stl raised the question whether this mechanism of action is conserved in all these repressors, or conversely, new strategies to sense their helper inducing phages exist in nature. To answer this question, we have resolved the X-ray structure of SaPI1 Stl in complex with its inducer, Sri protein from 80a phage. SaPI1 is one of the prototypical islands used to decipher the biology of the SaPIs and is clinically relevant by encoding the TSST-1 toxin. The analysis of this structure shows a new tetrameric family of repressors with a new and stronger repression-derepression system involved in the control and transfer of mobile genetic elements. Also, our results demonstrate that this new system is not exclusive of the SaPIs but widespread in nature.

A290

The synergistic effect of antibiotics and bacteriophage on antimicrobial resistance evolution in *Staphylococcus aureus* by transduction

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Abstract

Bacteriophage are both bacterial predators and significant drivers of antimicrobial resistance (AMR) evolution by transduction, the major horizontal gene transfer mechanism in *Staphylococcus aureus*. Phage are often present alongside antibiotics in the environment, yet the consequences of transduction in such settings are unknown. We aim to understand how antibiotics and phage may act in synergy to generate and select for multidrug-resistant bacteria via transduction.

We investigate an environment where two strains of *S. aureus* are present at stationary phase (10^9 bacteria/mL), each carrying either an erythromycin or tetracycline resistance gene. We adapt an existing mathematical model of phage-bacteria dynamics, including transduction, to add the pharmacodynamics of these two antibiotics, and generate *in vitro* data to parameterise this addition.

By simulating scenarios where phage and/or antibiotics are present at varying timings and concentrations, we find that phage and antibiotics lead to faster bacterial eradication together rather than alone. However, by reducing bacterial growth antibiotics prevent phage replication, as the latter relies on bacterial machinery. If a low concentration of phage is introduced shortly after antibiotics, multidrug-resistant bacteria may be generated by transduction and selected for by antibiotics. This is only prevented with a high initial concentration of phage (>10^{10}/mL) or antibiotics (>2mg/L).

Although multidrug-resistant bacteria may be cleared within 24h, they could further evolve or be transmitted to other individuals during this time. The risk for transduction to lead to AMR evolution should be carefully considered when phage and antibiotics are jointly present, whether in the environment or during antibacterial treatment.

A291

Validation of the expression of phage marker genes from the Liverpool Epidemic Strain of *Pseudomonas aeruginosa* using qRT-PCR

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Abstract

Pseudomonas aeruginosa is one of the major causes of morbidity in cystic fibrosis (CF) patients. *The* Liverpool Epidemic Strain (LES) is associated with more severe disease and is transmissible between hosts. Previous studies revealed five novel prophages in the LES genome and detected abundant free LES phages in CF patient sputum. Three active LES phages were isolated from LES cultures and found to confer fitness advantages to their host, but the specific mechanisms are not known. Our study aims to identify the molecular mechanisms by which the LES prophages impact the biology of the *P. aeruginosa* host.

We used a well-characterised laboratory strain of *P. aeruginosa* (PAO1) that is susceptible to lysogenic infection by three LES phages. This enabled the construction and whole genome sequencing of lysogen variants harbouring three LES prophages (specifically Φ2, Φ3 and Φ4) in all possible combinations, creating single, double, and triple lysogens. In the present study we validate functional annotation of each prophage by quantifying expression of key genetic markers for lysogeny as well as early and mid/late genes of lytic replication from the LES phages using qRT-PCR. These data will inform whole transcriptome studies under a range of conditions to identify how such prophages control infection-relevant phenotypes to enhance the fitness of their host cells in the CF lung.

IMPORTANCE: Our study shows that LES phages increase the fitness of their host through specific interactions. Further investigation in clinically relevant conditions could identify unique targets for tackling recalcitrant *P. aeruginosa* infections.

A292

Prophage encoded genes in *Staphylococcus aureus* contribute to biofilm formation in severe recalcitrant chronic rhinosinusitis.

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Abstract

Background:

Staphylococcus aureus chronic relapsing infections are correlated with the persistent severe inflammation of the upper airway mucosa leading to chronic rhinosinusitis with nasal polyps (CRSwNP). Prophages affect bacterial fitness in multiple aspects, help bacteria dominate a niche long term, and can contribute to chronic infections and inflammation. Here, we aimed to understand if *S. aureus* prophage is associated with virulence and disease severity.

Methods:

Prophages were identified using PHASTER. ResFinder and VF Analyzer were used to detect virulence and AMR genes respectively. Prophage from hyper-biofilm forming strain (CI333) was transduced into other clinical strains (CI222 and RN4220). Beta-hemolytic activity was tested and biofilm biomass and metabolic properties between strains were compared with crystal violet and alamarBlue assays.

Results:

Two *S. aureus* clinical isolates (CI222 and CI333) were isolated from the same severe recalcitrant CRSwNP patient 2.5 years apart. The core genetic make-up of the two CIs was similar, indicating them to be the same strain. Both strains contained a Sa2int prophage. Additionally, a ~50 kb prophage belonging to Sa3int group was integrated in CI333 only. This prophage harboured genes responsible for human immune evasion (*sak, scn, chp, sea/sep*) and was integrated into the beta-hemolysin gene site, disrupting the beta-hemolytic activity. The biofilm biomass of CI333 was significantly larger than that of CI222 and RN4220. Prophage IME1361_01 was successfully induced from CI333 and introduced into CI222 and RN4220. The newly lysogenized strains lost their beta-haemolytic activity and had a significantly higher biofilm biomass than their parent strains ($p < 0.05$).

A293

Conflict in the pangenome: characterizing the molecular basis of plasmid fitness costs and compensation across divergent species of bacteria

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Abstract

Horizontal gene transfer (HGT) is an essential component of bacterial evolution and ecology, facilitating the spread of significant traits including antimicrobial resistance. However, openness can be costly; incoming genes can introduce conflicts with resident genes, forming a barrier to HGT and thus bacterial adaptation. Previous work has identified the hypothetical chromosomal protein PFLU4242 as a key cause of plasmid-imposed fitness costs — and thus a major barrier to the maintenance of different pQBR plasmids — in *Pseudomonas fluorescens* SBW25. PFLU4242-pQBR plasmid interactions are therefore an exemplary model of pangenome conflict. Preliminary studies have shown homologues of this uncharacterised protein in diverse species, but its physiological activity and ecological function remain unclear.

We conducted sequence-based analyses to understand the distribution of PFLU4242 homologues across species and strains, and built phylogenies to determine its evolutionary history and association with other genes. Our data suggests that PFLU4242 is part of the accessory genome and is spread between lineages by HGT, though it does not appear closely associated with known mobile genetic elements. Given genomic co-localisation of PFLU4242-like proteins with known elements of 'defence islands', we hypothesise that PFLU4242-like proteins are a genome defence mechanism. Current work aims to determine the effects of PFLU4242-like proteins on bacterial fitness and plasmid receptiveness, and determine the underlying molecular basis of DUF262 interactions. Our work provides better understanding of plasmid/host dynamics and explores the poorly understood trade-off between openness to HGT and genome defence, thought to be a key mechanism determining genome content, adaptive capacity, and pangenome structure.

A294

Novel interactions of the CRISPR Cas1-Cas2 complex.

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Abstract

CRISPR-Cas systems provide protection for prokaryotes against mobile genetic elements (MGEs), such as bacteriophage. The Cas1-Cas2 protein complex is the highly conserved keystone of CRISPR-Cas adaptive immunity because it captures MGE DNA (or RNA if fused to a reverse transcriptase). After DNA capture, it is an integrase that expands the CRISPR locus with short fragments of DNA from the MGEs, building a record of encounters. This therefore establishes future immunity. A great deal of mechanistic detail is known about the structure and function of the Cas1-Cas2-DNA capture complex. But important questions remain – including how is the MGE DNA captured in the first instance? And what other proteins are required for this?

To gain answers to these questions we have re-purposed for use in *E. coli* the mammalian BioID2 technique, for proximity dependent protein labelling that facilitates identification of protein-protein interactions *in vivo*. We will describe the BioID2 toolkit for use in *E. coli*, and its application for Cas1-Cas2 interactions that occur during CRISPR adaptation. This has provided us with significant new insights into how DNA is captured during adaptation, and has expanded protein networks that interact with Cas1-Cas2 in *E. coli* cells.

B001

Synthetic biology for the controlled production of high-value compounds in Phaeodactylum tricornutum

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Abstract

In recent years, interest in microalgae as biotechnological hosts has increased rapidly. Compared to the classic model organisms for metabolic engineering, bacteria and yeast, the photosynthetic microalgae present a sustainable alternative for producing high-value compounds, such as diterpenoids. Microalgae have the additional advantage of producing the terpenoid precursor molecules, geranylgeranyl diphosphate (GGPP) and farnesyl pyrophosphate (FPP), as part of their native metabolism for photosynthetic pigments and membrane sterols, respectively. With potable water becoming a restricted resource, marine microalgae such as Phaeodactylum tricornutum are particularly attractive as they can grow on salt and wastewater. Engineering terpenoid production in P. tricornutum requires the introduction of terpene synthases and cytochrome P450 enzymes. Using a range of novel and established DNA parts based on the MoClo syntax we generated a variety of strains producing the diterpene manoyl oxide. We will present data on the effect of promoters, introns, and codon optimisation on transgene expression levels. Regulatory elements allow for tuneable transgene expression. Being able to control the production of diterpenoids may be beneficial for overall culture health due to a lower metabolic burden on the cells and shorter exposure to the diterpenoid itself. We demonstrated tuneable transgene expression by using a newly identified promoter. Additionally, we assessed the long-term expression of exogenous DNA in a time-course experiment. In conclusion, we highlight that P. tricornutum has great potential for synthetic biology approaches and show areas in which further research may help to enhance the production of diterpenoids in this microorganism.

B002

Investigating 3-Hydroxypropionate Metabolism in *Parageobacillus thermoglucosidasius* NCIMB 11955

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Abstract

Background: 3-Hydroxypropionic acid (3-HP) is a 3-hydroxy isomer of lactate and a promising platform chemical with numerous industrial applications. *Parageobacillus thermoglucosidasius* is a thermophilic bacterium with significant attraction as a chassis for the production of fuels and chemicals. In this study, the metabolism of 3-HP in *P. thermoglucosidasius* was investigated and manipulated as it represents a promising host for the production of 3-HP.

Results: When wild-type *P. thermoglucosidasius* was grown in the presence of 3-HP, it was observed to utilise the compound as the sole source of carbon and energy. Elimination of this undesired characteristic was required if biological production of 3-HP was to be engineered in this chassis. Accordingly, the genes encoding two putative (methyl) malonate semialdehyde dehydrogenase genes (*mmsA1* and *mmsA2*) were inactivated by deleting their entire coding regions, singly and in combination, using a newly developed CRISPR/Cas genome editing tool. Whilst the wild-type consumes the available 3-HP in 12 hours, the knock-out mutant strains *ΔmmsA2* and *ΔmmsA2ΔmmsA1* were unable to utilise 3-HP, even after 84 hours.

Conclusions: Similar growth profiles for *ΔmmsA2* and *ΔmmsA2ΔmmsA1* were observed which suggests *ΔmmsA2* might be responsible for 3-HP consumption in *P. thermoglucosidasius* NCIMB 11955. The created *ΔmmsA2* mutant strain represents an ideal chassis for 3-HP production.

Keywords: 3-Hydroxypropionic acid, *Parageobacillus thermoglucosidasius*, Malonate semialdehyde dehydrogenase, Metabolism

B003

Dynamic methods in describing the physiological response of bacteria to nanomaterials

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Abstract

Nanomaterials are often studied in bacterial models to assess their antimicrobial potential. Usually, these tests are performed with end-point methods, including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), agar well diffusion, or plate count assays. While CFU numbers or general toxicity can be assessed with such an approach, these methods do not provide insights into the dynamic nature of changes in bacterial viability or physiology, which is crucial in evaluating nanomaterials for processes in applied microbiology. Therefore, there is a necessity to propose other measures that will increase the sensitivity for comparing the activity of nanomaterials on bacterial cells.

In this work, we used a dynamic approach to study the physiological response of selected microorganisms to nanomaterials.

Escherichia coli ATCC® 25922™ and *Staphylococcus aureus* ATCC® 33591™, and *P. aeruginosa* ATCC®27853™ served as reference microorganisms. Carbon nanomaterials (i.e., nanotubes and graphene oxide plates) and metal oxide nanoparticles (i.e., TiO2, ZnO, Fe3O4) were tested with the bacteria. Bacterial physiology was studied with spectrophotometry (absorbance and fluorescence), phase-contrast and fluorescence microscopy, and flow cytometry. Morphology, respiration, metabolic activity (production of pigments), population growth kinetics, membrane potential, and antagonistic behavior were tested.

The dynamic approach has shown the effects of selected nanomaterials on bacterial physiology in greater detail than the end-point methods and indicated their advantages and disadvantages. For example, spectrophotometric methods were affected by the influence of nanomaterials (particularly those based on carbon) on optical density. However, applying biochemical assays and introducing a larger number of repetitions increased the precision of conducted experiments.

B004

The ammonia-oxidising bacterium *Nitrosomonas europaea* acquires ammonium using a Rhesus protein.

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Abstract

Ammonia oxidizing bacteria (AOB) oxidize ammonia to nitrite, it is the first and rate-limiting step in the important process of nitrification. Amongst the AOB, *Nitrosomonas europaea* is one of the most studied and understood bacteria, and while nitrification is a central part of its metabolism, little is known about the way it acquires ammonium from the environment. The genome of *N. europaea* encodes for an ammonium transporter named rhesus 50 (Rh50) which belongs to the ubiquitous Amt-Mep-Rh superfamily of ammonium transporters. While extensively researched, the transport mechanism of this crucial family of transmembrane proteins has been elusive and under intensive debate for years. In addition, despite their fundamentally divergent physiological role in bacteria, fungi, plants, and animals, Amt-Mep-Rh proteins are structurally very similar, raising the possibility of a conserved ammonium transport mechanism across the superfamily.

In this context, using yeast complementation assay and electrophysiology measurements, we propose to elucidate at the molecular level, the mechanism of ammonium translocation through rhesus protein. This will not only enable us to better understand the physiology of the *N. europaea* and its ability to use ammonium as an energy source but also has important medical implications, as malfunctions of Human rhesus proteins are associated with severe human pathophysiology.

B006

The discovery of recently evolved virulence factors reveals new insights into Type IV Secretion Systems mechanism

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Abstract

Bacterial secretion systems are crucial for host-pathogen interactions: bacteria utilize these essential membrane-embedded virulence apparatuses to translocate key weaponry into the host, to manipulate infection responses. The type IV secretion system (T4SS), particularly appealing as a drug target, transports macromolecules such as effector proteins and DNA (including antibiotic resistance genes). The bacterial pathogens *Legionella pneumophila* and *Coxiella burnetii,* the causative agents of Legionnaire disease and Q fever, respectively, employ similar T4SS to translocate a large effector proteins plethora into their hosts.

Recently, we have isolated from *L. pneumophila* the Type IV Coupling Complex (T4CC), an essential apparatus that recruits effector proteins to the T4SS, and determined its high-resolution Cryo-EM structure. Two previously unreported proteins, we named DotY and DotZ, co-eluted with the T4CC and were co-dependent: DotY deletion resulted in DotZ absence from the T4CC, and vice versa. Additionally, we found that DotY and DotZ deletion affects T4CC function and localization.

Intriguingly, *ab-initio* structural prediction of *cbu1754*, *C. burnetii's* gene important for intracellular replication, identified it as a homolog of LvgA (*L. pneumophila* T4CC component), despite no sequence similarity. We verified Cbu1754 function by co-purification with *C. burnetii's* T4CC and *in vivo* assays.

With no sequential homologs outside their genus, and located outside T4SS operons, these components are likely newly evolved T4SS members. We conclude that T4SS dynamic evolution suggests additional factors will be further discovered by untraditional genetic methods. A broader understanding of T4SS architecture and mechanism offers new strategies for novel drug development, essential in fighting infectious diseases.

B007

The effect of environmental pollutants on antibiotic resistance in Gram-negative bacteria

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Abstract

Antibiotic resistance is a major global threat to human health. Although the clinical implications of antibiotic resistance have been widely studied, little is known about the role of environmental chemicals in its development and spread. Antibiotics can enter the environment from a range of sources, including agricultural runoff and effluents from hospitals and pharmaceutical companies. Antibiotics in the environment often co-exist with other environmental pollutants, such as personal care products and pesticides. These pollutants may act as co-stressors to bacteria and exert selective pressures to potentially accelerate antibiotic resistance. However, the influence of co-stressors on the evolutionary trajectory of antibiotic resistance remains poorly understood. Therefore, the aim of this study is to explore the effect of environmental pollutants on the antibiotic resistance of Gram-negative bacteria using the clinically relevant strain, *Enterobacter cloacae* ATCC13047. Bacterial growth was analysed for short exposure (2 days) and prolonged exposure (5 days) to different combinations of environmental pollutant and antibiotic. We investigated 7 widespread environmental pollutants with 2 antibiotics and observed that prolonged exposure to sub-lethal concentrations of pollutant and antibiotic resulted in increased resistance. These results were found for 2 of the 7 environmental pollutants when combined with norfloxacin, to which ATCC13047 is sensitive. Our results suggest that environmental pollutants may aid the development of antibiotic resistance and open an area of research that has been underexplored.

B008

Is *Clostridium difficile* sweet for trehalose?

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Abstract

Clostridium difficile causes infection upon disruption of the gut microbiota allowing unoccupied nutrient niches to be capitalized upon. Trehalose is a disaccharide consisting of two glucose monomers bonded by an α 1,1 glycosidic bond and found widely in the diet as it is a common sweetener and preservative in food. Trehalose metabolism in *C. difficile* has been suggested to have led to the emergence of hypervirulent strains, namely the ribotype 027 and ribotype 078 strains, due to increased sensitivity to trehalose *in vitro*. Here the ability of different *C. difficile* ribotype strains, across the five clades, to utilize trehalose were investigated through growth and gene expression analysis. The R20291 (RT027) and M120 (RT078) strains showed robust growth as observed before, whilst the TL178 (RT002), CD305 (RT023) and CF5 (RT017) strains did not. Nested PCR showed that in the R20291 strain, the *treR* and *treA* genes are co-transcribed, whilst in the M120 strain, the *ptsT* and *treA2* genes are co-transcribed as well. Using RT-qPCR, expression levels of the *treA* and *treR* genes increased across all strains with the exception of the CF5 strain, whilst the secondary gene cluster genes, found in the M120 and CD305 strains, all increased as well. Trehalose is also shown to repress both toxin expression and sporulation in the R20291 and M120 strains. This data demonstrates the conserved ability of *C. difficile* to detect and respond to trehalose, however, utilization during *in vitro* growth differs marking obvious genetic differences between strains.

B009

Metabolic cycles are robust and respond to nutrient changes in single cells of budding yeast

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Abstract

The yeast metabolic cycle (YMC) is a biological rhythm present in budding yeast. This biological rhythm is linked to the cell division cycle and entails oscillations in oxygen consumption, intracellular metabolite concentrations, and cellular events. Most studies on the YMC have been based on bulk-culture experiments, and the behaviour of the YMC in individual cells is unclear. In particular, it is unknown both how nutrient changes affect the frequency of the cycles and what its underlying molecular mechanism is. Furthermore, it is unclear whether such cycles arise from interactions between cells or are generated by individual cells. I aim to characterise the YMC in single cells and its response to perturbations. Specifically, I use microfluidics to trap individual yeast cells and record the intensity of flavin fluorescence, a component of the YMC.

To analyse the thousands of flavin time series produced, I have developed a machine learning pipeline for classifying time series into oscillatory and non-oscillatory groups. I further show that autoregressive models can help extract the frequency components from time series data with low signal-to-noise ratio.

My results show that cycles of flavin intensity synchronise with budding in both prototrophic and auxotrophic strains. Furthermore, such cycles in single cells differ from oscillation of oxygen consumption in chemostats as suggested by previous studies. This observation calls into question whether diffusion of chemicals between cells is required for the YMC.

B010

Improving detection time of trimethoprim resistance in point-of-care antimicrobial screening for urinary tract infections

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Abstract

Urinary tract infections (UTIs) are largely caused by Uropathogenic *Escherichia coli* (UPEC) and nitrofurantoin has replaced trimethoprim as the first-line treatment due to rising trimethoprim resistance rates. However, there is currently a lack of viable point-of-care options for diagnostics and antimicrobial screening for UTIs and for bacteriostatic antimicrobial compounds which impede cellular growth, like trimethoprim, detection time for sensitivity is a prohibiting factor in rapid antimicrobial sensitivity screening.

ATP bioluminescence is a fast and inexpensive way of detecting live bacterial cells and has been used extensively for antimicrobial sensitivity screening. Trimethoprim sensitivity is currently detectable after 90 minutes using ATP bioluminescence. However, published works have indicated that adding certain amino acids to the assay may improve trimethoprim speed of action. Here we aimed to understand the mechanisms behind the ATP response during trimethoprim challenge.

We obtained trimethoprim sensitive and resistant clinical UPEC strains from our local primary care facility (Norfolk and Norwich University Hospital) and used high-throughput techniques, including the Seahorse Analyzer (Agilent) and CLARIOstar Plus (BMG Labtech), to identify patterns in respiration and intracellular ATP over time, respectively. These patterns have informed our approach to an *in silico* analysis of the pathways affected by trimethoprim, using genome-scale metabolic modelling to understand its effect on intracellular ATP concentration. We have also collected >200 UPEC isolates from UTI patients to validate new cut offs for trimethoprim sensitivity using ATP bioluminescence. Reducing the detection time of trimethoprim sensitivity (in line with other antibiotics) will expand treatment options offered by point-of-care UTI testing.

B011

Transmorgification of anomalous sources for regulation of analytical mechanisms in microorganisms

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Abstract

In presence research our direction relate with diverse microorganism by execution of microbiological techniques by analysis communication of behaviour of strains in sense of interaction of lineage of sources in relation of human for combating infections by leading best modernise ways. Exacerbation of research over the eras has stuck learners to consider towards more appropriate methods as fascilitators with reference of highly caliber constitution in form of literature by ranking research as a source of linker of methods from simple towards complex research innovation. Microorganisms with different morphological or biochemical behaviour was consider as model of studies for comparison of analytical regulatory mechanisms. Apropos assays was speculate with different situations with requisition of interaction of vital applications relate with specific species behaviour cultures. Transformation of ionisation of metal metabolism or immobilisation was consider as modifier for biotransformation in presence of dinitrogenase reductase or ADP .NAD was analyze as initial parameter for ADP ribosylation . Transformation was excogitate with exclusion of ATP, NADPH ,NADP , or inorganic phosphate water. As Microorganisms constitute definitud sensors in their messenger RNA for regulations of metabolites for expedient objectives for accentuating of gene expression. Biotransformation was taken into envisage how ionisation of various monetise exist in tonality from anomalous towards necessities chains of physiological purposes for regulations of target product by modelling of bacteria as secondary messenger. Our concentration about specialisation in comparison of microbiology based on several circles restricted to theorize research reflections influences with diverge sections as learning approaches as basic objective for economically embellishments.

B012

Investigating the Role of Tryptophanase in *E. coli* Biofilms

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Abstract

Tryptophanase (TnaA), an enzyme present in many species of bacteria, is best known for the conversion of L-tryptophan to indole, pyruvate, and ammonia. However, TnaA can also metabolise other amino acids. We recently observed that a clumping phenotype seen during the exponential growth phase of a uropathogenic *E. coli* strain (ATCC® 25922™) was absent in a *tnaA* knock-out. We also found that this phenotype, which is characteristic of early-stage biofilm development, was reduced by novel TnaA inhibitors. Using clinical isolates from urinary tract infections (UTIs), which produce biofilms with indwelling catheters, we developed an assay to demonstrate that these compounds also reduce their biofilm development. The compounds are of interest because they could lead to the use of a TnaA inhibitor as a combination therapy with existing antibiotics to mitigate recurrent UTIs and biofilms. It was originally assumed that the absence of clumping in the mutant was due to the loss of indole production since previous studies have indicated that indole influences biofilm development. Upon closer inspection, we found that the absence of L-tryptophan in growth medium had no effect on the phenotype, but the absence of L-arginine prevented clumping. Furthermore, the addition of indole to the growth medium of the *tnaA* knock-out did not restore clumping. It remains to be seen which metabolite of L-arginine is responsible for this phenotype. These observations shed new light on the role of TnaA and suggest a route towards a more comprehensive understanding, and therefore treatment, of biofilm formation in uropathogenic *E. coli*.

B013

Control of bacterial flotation, gas vesicle biogenesis, motility, antibiotic production and virulence through XynR

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Abstract

The opportunistic pathogen *Serratia* sp. ATCC 39006 (S39006) is a rod-shaped, motile, Gram-negative bacterium that produces a β-lactam antibiotic (a carbapenem) and a bioactive red-pigmented tripyrrole antibiotic, prodigiosin. It is also the only known enterobacterium which naturally produces intracellular gas vesicles (GVs), enabling cells to float in static water columns. Regulation of GVs and secondary metabolites in S39006 can be coordinated but such pleiotropy is still poorly understood. To uncover novel inputs to this complex regulatory network, we used transposon mutagenesis to identify a mutant with an insertion in the IclR-type transcriptional regulator gene, *xynR*. The mutant showed diminished production of the carbapenem, prodigiosin, GVs and cellulase. Furthermore, the mutant also showed increased flagellar motility but exhibited attenuated virulence *in planta* and against *C. elegans*. Using differential expression analysis of the intracellular proteomes of the wild type and *xynR* mutant, we confirmed that the mutation negatively impacted expression of the corresponding GV, carbapenem and prodigiosin gene clusters. Conversely, flagellar and chemotaxis proteins were overexpressed, consistent with the increased motility of the mutant. We also found that the proteins encoded by a putative *yagEF-yjhF* operon, involved in xylonate catabolism and transport, showed a 32- to 128-fold increase in expression. We show that *xynR* is a repressor of xylonate catabolism in S39006 and suggest that xylonate is potentially involved in controlling carbapenem and prodigiosin biosynthesis. Our results indicate that XynR is a global regulator that controls antibiotic biosynthesis, flotation through modulating GV assembly, and has pleiotropic impacts on the physiology and virulence of S39006.

B015

Altering *adeABC* Antibiotic Sensitivity Response with Quadruplex DNA

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Abstract

Quadruplexes are non-canonical nucleic acid structures formed from G-rich sequences of DNA or RNA strands. These complex and stable structures have been involved in significant biological functions, including regulating gene expression within microbes. A potentially significant application of quadruplex therapeutics is in antimicrobial resistance (AMR). This study identified the potential role of quadruplexes in AMR within the *adeABC* efflux pump genes of *Acinetobacter baumannii*.

Antibiotic sensitivity profiling studies in forty-three clinical *A.baumannii* strains revealed multi-drug resistant properties to Ertapenem, Meropenem, Tetracycline and Ciprofloxacin. Role of the efflux pump genes, *adeABC*, to the resistance observed was further confirmed by an inhibitory assay in the presence of PaβN. The downregulation by PaβN, with a 97.8% decrease in bacterial growth, combined with ertapenem indicated the significance of *adeABC* in AMR. Therefore, quadruplexes having similar function of downregulating the mechanism of efflux genes in resistance introduces a viable alternative in developing a novel antimicrobial therapy.

Putative Quadruplex-forming Sequences (PQSs) were identified within the efflux pump gene using a predictive software. The PQSs were assembled under specific conditions via heat denaturation techniques in ion buffers and analysed via UV-Vis Thermal Difference Spectroscopy (TDS) to identify successfully formed quadruplexes. Five of the seventeen generated PQSs evidenced results consistent with quadruplex formation.

The anticipated finding from this study is distinctive in its ability to be translated into clinical settings. Targeting the action of these physiologically active structures may inhibit resistance mechanisms and restore efficacy of many antimicrobial chemotherapies.

B016

The making of a superbug: Understanding the mechanism of high-level antibiotic resistance in *Staphylococcus aureus*

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Abstract

The incidence of both clinical and community-acquired *S. aureus* infections resistant to all classes of antibiotics necessitates new research to understand antibiotic resistance. Despite previous research the precise mechanism that allows penicillin binding protein 2a (PBP2a) to support continued cell growth and division in otherwise inhibitory concentrations of antibiotics is not yet understood. This research aims to delineate how *S. aureus* maintains high level antimicrobial resistance and the role of PBP2a and its interactions with the respiratory chain and cell wall components.

This study uses an optimised bacterial two hybrid assay to identify novel protein-protein interactions between cellular components and chosen proteins of interest, with roles in antimicrobial resistance and the maintenance of cell wall homeostasis. The bacterial two hybrid assay was used to identify novel protein-protein interactions between PBP1, PBP2, PBP2a, and cellular components. A library screen was optimized and over 23,000 clones containing potential prey/interacting partners of each PBP were screened. 10 novel interacting protein fusions were identified from a random genomic library. In tandem, a more targeted approach was used to screen an array of previously developed protein constructs against bait proteins of interest, identifying six further protein-protein interactions.

None of these interactions have previously been identified in *S. aureus*. While further investigation using entire genes fused to reporters and the Luciferase live cell assay system is required in order to localise and further characterise these protein-protein interactions in *S. aureus*, this study helps develop understanding of the mechanism of cell growth in the presence of antibiotics.

B017

The conserved mycobacterial penicillin binding protein LpqF is important for colony morphology and cell size.

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Abstract

Mycobacterial peptidoglycan stands out amongst other bacteria due to its unique structure and biochemical context. Many mycobacteria are human pathogens that use a range of effectors and cellular structures to evade or modify host responses and survive within their niche. A critical component of the mycobacterial cell envelope is peptidoglycan. This scaffold-like layer is made up of glycan chains, fastened together with interconnecting peptide bridges. Peptidoglycan must be rigid to support osmotic pressure of the cell and thus provides cell shape. The mycobacterial cell wall is also dynamic allowing the peptidoglycan to undergo significant maturation as the bacteria grow and divide, including alterations to the degree and nature of peptidoglycan cross-linking. Changes within this ultrastructure often give rise to emergent properties like colony morphology. We conducted a *Tn*-screen in *Mycobacterium marinum* to identify genes contributing to colony morphology and filtered the hits for those likely to act upon the cell wall. In this study we investigated the function of the previously unstudied mycobacterial penicillin binding protein, LpqF. We have shown that *lpqF* contributes to cell size and shape in *M. marinum* and *Mycobacterium smegmatis*. We show that despite its annotation the enzyme is not a β-lactamase but that it does have a canonical PBP-fold with an appended NTF2 domain. Intriguingly our crystal structure suggests that the NTF2 domain acts as an auto-regulatory domain in the protein. Furthermore, our results suggest LpqF undergoes cleavage in acidic conditions. This study identifies a role for LpqF in cellular morphology and mycobacterial peptidoglycan structure.

B018

An AraC-type transcriptional regulator modulates antibiotic production in *Serratia*

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Abstract

Antimicrobial resistance (AMR) is an increasing problem, exacerbated by global dissemination of drug resistance genes under selection pressure. Moreover, the rate of new antibiotic discovery declined over previous decades and so there is a growing need for antibiotic discovery and a deeper appreciation of the various genetic and physiological factors that influence antibiotic biosynthesis.

The enterobacterium *Serratia* sp. ATCC 39006 (S39006) is a useful Gram-negative model for studies on the biosynthesis and regulation of bioactive secondary metabolites, particularly two antibiotics - a carbapenem and prodigiosin. Both compounds are tightly regulated in response to various physiological and environmental signals, including quorum sensing.

We describe an AraC-family transcriptional regulator, a VfmE homologue, which modulates antibiotic production in S39006. The corresponding mutant showed reduced production of antibiotics, modulated at the transcriptional level of the corresponding biosynthetic operons. Furthermore, diminished cellulase production and enhanced motility also arose, confirming significant pleiotropy. Exploiting phage transduction, we constructed 15 double mutants with known S39006 regulators to infer connections to the wider regulatory network, where the *vfmE* phenotype was epistatic over several, consistent with a global role. Functional characterisation of the *vfmE* mutation and its physiological impacts in the modulation of antibiotic production and in wider pleiotropy were dissected. Mechanistic understanding of the regulatory processes involved may prove exploitable in enhancing controlled antibiotic hyperproduction.

B019

Environmental feedback and short-range cell interactions shape the spatio-temporal dynamics of the bacterial oxidative stress response

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Abstract

Bacterial phenotypic heterogeneity is a hallmark of stress response. Noisy stress responses are often attributed to the stochastic processes underlying gene expression. Here, we investigated the response to oxidative stress, which bacteria encounter as a result of aerobic life and infection. Using microfluidics-based single-cell imaging of *E. coli*, we found that OxyR-regulated gene induction in response to constant and uniform hydrogen peroxide exposure was highly variable across individual cells. Surprisingly, a machine-learning model was able to predict the variable amplitude of the stress response with high accuracy, showing that the apparent intracellular noise in fact has a deterministic origin. This approach allowed us to decouple intracellular noise sources from variation in the environment, cell morphology, and the effects of cell lineage history. By querying the machine-learning model, we were able to identify deterministic features accounting for the high predictive power. Specifically, the oxidative stress response of an individual cell was largely governed by the number of neighboring cells and their spatial arrangement. We tested this prediction using genetic perturbations and by varying the density and structure of cells within microfluidic chips and in bacterial colonies. Under stress, induction of H_2O_2 scavenging enzymes via the OxyR response strongly reduces the H_2O_2 concentration in the local environment, leading to inter-cellular feedback and cross-protection between neighboring cells. Therefore, what at first glance appears to be noisy gene regulation is in fact a precise response that is tightly coupled to a dynamic environment.

B020

The rise and fall of antibiotic tolerance: the role of *mexS* and *mexT* in *Pseudomonas aeruginosa*

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Abstract

Removal of toxins is key to bacterial survival and is achieved through efflux in *Pseudomonas aeruginosa*. Expression of the efflux pump MexEF-OprN, regulated by MexS and MexT, increases efflux of antibiotics and therefore an antibiotic-resistant phenotype. Under selective antibiotic pressure, mutations within *mexS* cause continuous production of MexT and thus resistant phenotypes. After removal of antibiotic selection, resistant mutants revert to sensitive phenotypes via mutations in *mexT* as opposed to re-functionalisation of *mexS*. We investigated the phenotypic impact of mutations in *mexS* and *mexT*. Exposure to 400µg/ml chloramphenicol selected strains with increased chloramphenicol tolerance, which were then passaged in chloramphenicol-free media. Colonies were replica plated with and without 400µg/ml chloramphenicol identifying strains reverting to reduced chloramphenicol tolerance. Two *P. aeruginosa* strains produced three resistant mutants with increased chloramphenicol tolerance which proceeded to generate five revertants with reduced chloramphenicol tolerance. Genome sequencing confirmed mutations in *mexS* resistant mutans and mutations in *mexS* and *mexT* in revertants. Using growth curves as a proxy for fitness, all three resistant mutants showed increased fitness over their respective parent and revertant strains in the presence of chloramphenicol, and reduced fitness in its absence. Differences in fitness between parent and revertant strains was varied according to it genotype and chloramphenicol presence being either more, less, or of equal fitness. These data indicate that reversion to a more sensitive phenotype via a *mexS/mexT* double mutation rather than the wild-type genotype cannot be solely explained by increased fitness caused by the double mutation.

B021

Streptomyces venezuelae peptidases with a putative role in resistance to peptide antibiotics

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Abstract

Antimicrobial peptides (AMPs) are a promising source of antibiotics, potentially effective against multi-drug resistant bacteria. Whilst AMP resistance among low GC-content Gram-positive bacteria has been studied in detail, the situation in high-GC Gram-positives is poorly characterised. Here, we identify that *Streptomyces venezuelae* is highly resistant to the AMP bacitracin with an MIC of approximately 64 µg/ml, compared to 0.5-4.0 µg/ml in other *Streptomyces* species. Transcriptome analysis of *S. venezuelae* found many putative peptidase-encoding genes strongly up-regulated under bacitracin challenge, leading us to propose that AMP degradation may contribute to the observed resistance phenotype. Using both a bioassay and HPLC quantification, we could show that cell suspensions of *S. venezuelae* rapidly degrade bacitracin. Subsequently, we were able to demonstrate that degradative activity resided in the culture supernatants indicating that bacitracin degradation was an extracellular phenotype of *S. venezuelae*. Indeed, *S. venezuelae* was able to protect sensitive *Streptomyces* species from bacitracin inhibition on solid medium, implicating bacitracin-degrading peptidases in microbial community interactions. Comparison of 22 bacitracin-regulated extracellular *S. venezuelae* peptidases - predicted using the MEROPS database - with experimental bacitracin resistance for 12 *Streptomyces* species identified the abundance of five S12 family peptidases as a strong predictor of resistance. S12 peptidases are therefore the most likely candidates for degradation-mediated bacitracin resistance in *S. venezuelae.* We therefore propose that degradation of AMPs may be a widespread mechanism of resistance in *Streptomyces* species with a putative role in protection of sensitive soil microbiome inhabitants from AMPs.

B022

Burkholderia cenocepacia small heat-shock proteins play a role in antibiotic heteroresistance and virulence

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Abstract

Burkholderia cenocepacia is a multidrug resistant pathogen that displays heteroresistance to bactericidal antibiotics by mechanisms not well understood. Exposure to sublethal proteotoxic stress (e.g., heat, peroxide, and the antibiotic streptomycin) in *Escherichia coli* induces intracellular protein aggregates (PAs) localized to the bacterial poles. PAs are subject to limited disaggregation after removal of stress and the cells inheriting the ancestral PAs are better able to cope with subsequent exposure to proteotoxic stress. Small heat shock proteins of the Hsp20 family are associated with the appearance of PAs, as they bind misfolded proteins to prevent irreversible protein aggregation. We hypothesized that asymmetrically inheriting PAs from parental cells during cell proliferation could lead to heteroresistance. We identified three genes encoding Hsp20 family proteins in *B. cenocepacia*: hspA(BCAL1233), hspB(BCAL1234), and *hspC* (BCAM0278). Single and combination deletion mutants were examined by population analysis profiling and E-test against polymyxin B (PmB) and ceftazidime (CFZ). We observed that Δ*hspABC* displayed significantly reduced heterogeneity in response to both antibiotics. Complementing the mutant with any of the single genes did not restore heteroresistance. In comparison to wild type and the other mutants, Δ*hspABC* mutant cells under antibiotic stress showed higher levels of membrane lipid-peroxidation and evidence of damaged cell membranes. Both Δ*hspC* and Δ*hspABC* showed similar levels of reduced virulence in the *Galleria mellonella* infection model, suggesting *hspC* but not *hspA* and *hspB* is involved in virulence. Together, our results suggest that the three Hsp20 proteins play a critical role in determining antibiotic heteroresistance against bactericidal antibiotics.

B023

Investigation of the Localisation of FtsZ in Pathogenic *E. coli* upon Bacteriophage Addition in a Human Cell Model as a Biomarker for Antibacterial Agents

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Abstract

Antimicrobial resistance is a growing problem worldwide and has created a need for novel antibacterial agents and strategies. *Escherichia coli* is one of the most common Gram-negative pathogens and is responsible for infection leading to neonatal meningitis and sepsis. The FtsZ protein is a bacterial tubulin homolog required for cell division in most species, including *E. coli*. Several agents that block cell division have been shown to mis-localise FtsZ, including the bacteriophage **λ**-encoded Kil peptide, resulting in defective cell division and a filamentous phenotype. Therefore, FtsZ may be an attractive target for new antimicrobials. In this study we have developed an *in vitro* meningitis model system for studying the effect of bacteriophages on FtsZ using fluorescent *E. coli* EV36/FtsZ-mCherry and K12/FtsZ-mNeon strains in the hCMEC human brain cell line. We show localisation of FtsZ to the bacterial cell midbody as a single ring during normal growth conditions in the absence and presence of human cells, and mislocalisation of FtsZ producing filamentous bacterial cells upon addition of the known inhibitor Kil peptide. We also show that when bacteriophages K1F-GFP and T7-mCherry were applied to their respective host strains, these phages can inhibit FtsZ and block bacterial cell division in the exponential growth phase, leading to filamentous multi-ringed cells. Finally, we show that the T7 peptide gp0.4 is responsible for inhibition of FtsZ in K12 strains by observing a phenotype recovery with a T7Δ0.4 mutant.

B024

An 'Omics-based approach to identifying novel biocatalysts and metabolites

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Abstract

The activity of many enzymes expressed recombinantly in *Escherichia coli* cannot be determined using standard biochemical assays, as often the substrates that these enzymes are tested against are not relevant, or the products produced are not detectable. However, today the biochemical footprints of such unknown enzyme activities can be revealed via analysis of metabolomes of the recombinant *E. coli* clones employed, using sensitive technologies such as mass spectrometry (MS). By analysing the cell free extracts of a panel of clones, not only can the conversion of metabolites into novel, useful products be observed (with no specific precursor molecules added), but over-produced, potentially high- value metabolites, can be identified. In order to get as high a coverage of the potential metabolites present within *E. coli* as possible, a range of different extraction solvents have been used with the CFEs to find complimentary, high yielding extraction methods. These extraction methods with wide metabolite coverage, in combination with a wide panel of biocatalysis enzymes and chemometric analysis, any novel compounds can be identified. Eventually, once any unique metabolites have been identified, knock in/out studies can be performed to elucidate the biosynthetic pathways which are affected by the cloned in enzyme. The metabolome can then be further engineered to produce economically viable quantities of the high-value chemical, and purification techniques can be exploited to turn it into a final product.

B025

Some like it hot (or not); Ancestral niche limits bacterial adaptation to novel temperature conditions

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Abstract

Bacterial species are difficult to define; nevertheless, we often see bacterial genotypes cluster into clear phylogenetic "clades" with distinct ecological niches. In some Gram-positive groups, such as *Bacillus* and *Streptomyces*, clades are sometimes distinguished by thermal niche. Determining why thermal adaptation is associated with clade may improve our understanding of how phylogenetic groups remain coherent despite horizontal gene transfer. We conducted a selection experiment to test whether thermal niche constrains evolvability in *Bacillus cereus* species. Cold adaptation in *Bacillus cereus* has been associated with two genes, therefore we hypothesized that thermal niche is plastic and predicted that *Bacillus cereus* species would show greater fitness increases in response to novel temperature regimes. We evolved replicated lineages descended from a mesophilic or psychrotolerant *Bacillus cereus* species under "mesophilic" and "psychrotolerant" temperature regimes for over 700 generations. We determined fitness changes in evolved lineages using competition assays and conducted whole-genome sequencing to identify genetic variants associated with different treatments. Contrary to predictions, *Bacillus* lineages did not show consistent fitness increases in novel temperature regimes, but showed large fitness increases under ancestral temperature conditions. Rates of synonymous mutation were consistent across treatments, meaning that greater fitness increases were not caused by greater availability of mutations. Thermal niche may be a complex polygenic trait; this would lead to high levels of constraint and would explain its association with speciation in the *Bacillus* group. Gene knockout and knock-in experiments will be conducted to determine whether acquisition of novel genes via HGT can overcome thermal niche constraint.

B027

A novel efflux pump involved in polymyxin B heteroresistance in *Enterobacter bugandensis*

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Abstract

The *Enterobacter cloacae* complex (ECC) consists of Gram-negative bacterial opportunistic pathogens causing serious hospital-associated infections. Many ECC species are multidrug resistant, and their infections require treatment with last-resource antibiotics such as colistin or polymyxin B (PmB). However, colistin/PmB resistant and heteroresistant strains complicate the treatment in infected patients. While several mechanisms of colistin/PmB resistance have been elucidated (1), little is known about heteroresistance within ECC species (2). Here, we studied two *Enterobacter bugandensis* clinical isolates, an ECC specie that exhibits high levels of intrinsic PmB resistance (MIC from 512 to >1024 µg/mL). RNA-seq data revealed a novel efflux pump highly overexpressed in the presence of PmB in both isolates. This pump involves an AcrB homologue, a small hypothetical transmembrane protein, a porin and a lipoprotein. Mutants in the *acrB* and the hypothetical protein genes showed a lower level of PmB resistance and heteroresistance than the wild type when tested by both population analysis profile and Etest. Further, overexpression of both genes in a PmB sensitive *Enterobacter ludwigii* isolate (MIC < 4 µg/mL) that contains the other two components, and in *Escherichia coli* K12 (lacking all the four components) increased the level of resistance, especially when both genes were overexpressed together. Resistance in *E. ludwigii* was as high as in *E. bugandensis.* We conclude that the AcrB homologue and the small transmembrane protein are key components of a novel PmB resistance mechanism in ECC isolates expressing high levels of intrinsic PmB resistance and heteroresistance.

1. Zong *et al*. Trends in Microbiology. 2021-doi:10.1016/j.tim.2020.12.009.
2. Mushtaq *et al*. J Antimicrobial Chemotherapy. 2020-doi:10.1093/jac/dkaa201.

B028

Characterisation of the telomere maintenance protein mtc3, a yeast homologue of mitochondrial cytochrome oxidase subunits

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Abstract

A homologue of the cytochrome oxidase subunit COX5A/B (S.Cerevisiae) and COXIVA/B (H.sapiens) has been identified using bioinformatic analysis. MTC3 was identified in a genome wide screen as causing synthetic loss of fitness with a CDC13 mutant involved in the maintenance of telomere capping (MTC). Through a genome wide analysis of protein localisation, MTC3 was shown to have a mitochondrial localisation. Bioinformatic analysis has identified that this gene bears similarity to COX4/5 genes in other fungi. COX4/5, depending on the organism, is a subunit of cytochrome oxidase regulated by oxygen tension that alters affinity for oxygen in cytochrome oxidase in the last step of the electron transport chain. MTC3 has a predicted mitochondrial localisation motif at the amino terminus and a basic isoelectric point supporting the localisation of this protein in mitochondria. The identification of this mitochondrial gene in a screen as a regulator telomere maintenance presents an intriguing example to study the communication mediated between mitochondria and the nucleus. We will verify cellular localisation, effects on respiration of mitochondria and expression of cytochrome oxidase subunits using Saccharomyces cerevisiae in wild-type, deletion and mutant strains.

B029

The regulatory roles of Clostridioides difficile anti-sigma factor, rsbW, in stress management and infection.

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Abstract

Clostridioides difficile, a spore-forming, anaerobic bacterium is a primary cause of antibiotic-associated diarrhoea in developed countries. *C. difficile* faces a variety of environmental stresses during infection. To cope with these insults, it uses alternative sigma factor B (σ^B) to mediate transcription of several stress-dependent genes. σ^B is regulated and sequestered by anti-sigma factor, rsbW.

To understand the role of rsbW in *C. difficile* physiology, a rsbW mutant was generated, which allows us to assess the role of a post-translationally unbound σ^B in an "always on" manner. As σ^B does not regulate its own expression in *C. difficile*, a deleterious fitness defect was not observed. The rsbW mutant can tolerate acidic environments and detoxify reactive oxygen and nitrogen species compared to the parental strain. ΔrsbW also had a severe defect in spore formation and biofilm production. Interestingly, the rsbW mutant demonstrates an increased ability to adhere to the gut epithelium, as demonstrated in an *in vitro* human gut model, although the mutant was less virulent in a *Galleria mellonella* infection model. Notably, qPCR analysis of the mutant revealed a distinct upregulation of σ^B-controlled genes, as well as genes that are not directly controlled by σ^B, e.g. the pleiotropic transcriptional regulator sinR. A transcriptomic analysis is underway to investigate a potential broader role of rsbW in *C. difficile* gene regulation.

Our data suggests that rsbW plays a role in colonisation and persistence of *C. difficile*; rsbW may have a potentially exciting σ^B-independent role, which needs further investigation.

B030

Copper exposure triggers a metabolic shift in *Staphylococcus aureus* USA300

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Abstract

Metal homeostasis and metal resistance are important mechanisms required by pathogenic bacteria to infect and survive within a host. Copper is a highly toxic metal that is utilised by the innate immune system for its antimicrobial properties, accumulating at sites of infection and acting within macrophages to kill engulfed pathogens. We have identified a novel copper resistance locus comprising a P_{1B-3}-ATPase efflux transporter, CopX, and a novel lipoprotein, CopL, that confers copper hyper-resistance in the highly virulent community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) USA300. Importantly, the *copXL* locus plays a key role in intracellular survival within macrophages. To investigate the mechanisms involved we carried out RNAseq analysis of both *S. aureus* USA300 and an isogenic *copL* mutant in response to sub-inhibitory copper concentrations used in the macrophage assays. Our data show that copper exposure in *S. aureus* USA300 triggers a shift to anaerobic respiration, affecting the TCA cycle and fermentation pathways, interestingly linking copper homeostasis with central metabolism in this important human pathogen.

B031

Chance and Necessity: Using a Long Term Evolution Experiment to guide and understand routes for antibiotic strain improvement

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Abstract

Streptomyces, hyphal bacteria, are prolific producers of antibiotics that we exploit in human medicine (responsible for more than two-thirds used in the clinic). Understanding how these bacteria evolved and the regulation of antibiotic biosynthesis is key strategy in the fight against Anitmicrobial resistance. Evolutionary adaption is driven by the accumulation of mutations, but the temporal dynamics of this process are difficult to observe directly in end-point analysis. Using a combination of Long-Term Experimental Evolution (LTEE), molecular genetics and genomics we have begun studying how the adaptive mutations shape the genome of *Streptomyces coelicolor* M1152, when and in what order. Our LTEE consists of growing M1152 in liquid culture, with a total of 6 lineages. At generational milestones genomic and phenotypic characterisation was carried out and DNA from the community was genome sequenced. Following the reintroduction of the actinorhodin biosynthetic gene cluster, representatives were sequenced to assess the impact on antibiotic production. We see both phenotypic and genotypic alterations. Increased hyphal fragmentation in liquid culture, markers of both developmental cessation and progression on solid-surface media, an accumulation of small mutations across the genome and examples of increased/reduced/loss of actinorhodin production. Morphological adaption to liquid culture (increased fragmentation) is a desirable trait for fermentation. Developmental alterations are less linear than we would expect. The range of antibiotic production changes highlights the many different paths that impact antibiotic production. These results are promising for the future of the experiment as we begin looking at the effects on antibiotic production and metabolic adaption.

B032

Metabolites tune the antimicrobial susceptibility of Mycobacterium tuberculosis

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Abstract

Tuberculosis (TB) is a chronic bacterial infection, caused by *Mycobacterium tuberculosis (Mtb),* killing millions worldwide each year. With the increasing incidence of antibiotic resistance as well as Mtb's ability to enter a phenotypically dormant and drug tolerant state, efforts to control the disease are met with challenges. Therefore, it is an urgent need to develop new antibiotics and strategies to combat that antibiotic resistance arisen from current treatment regimens. Whilst elucidation of new potential drug targets is key, an attractive strategy for treatment is the identification of compounds that potentiate the activity of pre-existing antibiotics or even reverse resistance; thereby extending their efficacy and potentially shortening the lengthy treatment regime.

There is strong evidence that the metabolic state of Mtb impacts the efficacy of antibiotics and therefore modulating bacterial metabolism may improve antibiotic treatment. Determining metabolites that can potentiate drug treatments could facilitate the development of adjuvants for anti-TB therapy. Equally, identifying metabolites which protect Mtb against drug activity informs studies on drug tolerance thereby improving future drug discovery. Focusing on key metabolites in central carbon metabolism we have demonstrated that exogenous supplementation with specific metabolites can potentiate antibiotic treatment *in vitro*. These results demonstrate a viable strategy for potentiating antibiotic treatment against tuberculosis.

B033

The differential role of the phoPR system between *Mycobacterium tuberculosis* and *Mycobacterium bovis* and consequences in lipid biosynthesis.

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Abstract

Tuberculosis disease is caused by different bacterial species belonging to the clonal group named Mycobacterium tuberculosis complex (MTBC) that affect a wide variety of mammalian hosts. The MTBC can be mainly separated into two different groups, the human-adapted strains, and the animal-adapted strains. One key protein system of the MTBC is the PhoPR system, which is a two-component signal transduction system that directly regulates more than 30 genes of the mycobacterial genome and controls the biosynthesis of virulent lipids and secreted proteins.

The phoPR allele of the animal-adapted strains contains a two-nucleotide mutation compared to the human-adapted strains that might play a role in host preference. We hypothesized that the phoPR mutation occurring in the animal-adapted strains is actually advantageous for animal adaptation of the MTBC members by favouring a specific lipid biosynthetic pathway that modulates macrophage response. To test this hypothesis, we are using the reference strains *M. tuberculosis* H37Rv and *M. bovis* AF2122/97 as representatives of the human and animal adapted strains respectively. *M. bovis* phoPR knock-out mutants have been constructed and complemented with both the human and bovine allele of phoPR and RNA sequencing analysis has been done on those to give new insights on how the PhoPR system differentially modulates the genetic expression in human and animal adapted strains. Additionally, in-vitro characterization of these mutant strains such as growth in neutral and acidic conditions and lipid analysis has also been done.

B034

Chromobacterium violaceum encodes a functional quorum sensing-regulated type VI secretion system required for bacterial competition

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Abstract

The Type VI Secretion System (T6SS) is a contraction-based nanomachine used by many Gram-negative bacteria to inject toxic effectors inside adjacent cells. In this work, we characterized the T6SS of *Chromobacterium violaceum*, an environmental pathogenic beta-proteobacterium. We found that *C. violaceum* has a complete set of genes encoding the T6SS core components located in a major genomic cluster and six copies of *vgrG* distributed in the main cluster and four minor clusters. Using T6SS null mutant strains, western blot assays of secreted Hcp, and fluorescence microscopy of VipA fused to sfGFP, we showed that the *C. violaceum* T6SS is functional and required for competition against many Gram-negative bacteria, but has no role in *C. violaceum* virulence. Despite having high sequence similarity, the six VgrGs show little functional redundancy, with VgrG3 showing a major role in the T6SS function. Our data of co-immune precipitation favor a model of VgrG3 assembling heterotrimers with the other VgrGs. We determined that the expression of T6SS genes increased at high cell density on the dependence of the quorum sensing regulator CviR. Indeed, a Δ*cviR* mutant was completely defective in interbacterial competition, Hcp secretion, and T6SS activity. However, the Hcp protein levels remained unaltered throughout the growth phases, and the Hcp secretion decreased at high cell density, suggesting a complex regulation of the T6SS in *C. violaceum*. Overall, our data reveal that *C. violaceum* relies on a cell density-regulated T6SS to outcompete other bacteria and expand our knowledge about the redundancy of multiple VgrGs.

B035

Bacteriophage and their tail-associated lysins of Diabetic Foot Ulcer AMR pathogens

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Abstract

Diabetic foot ulcers (DFUs) are a significant complication of diabetes mellitus and often lead to lower extremity amputations due to the associated infections. These infections are polymicrobial and often dominated by *Staphylococcus aureus* and coagulase-negative staphylococci such as *Staphylococcus epidermidis*, which can form biofilms. In recent times there has been an increase in research into bacteriophages (phages) and their hosts, with a view to replace antibiotics with phage therapy. Novel antimicrobial approaches seek to exploit phage products and their mechanisms rather than using the entire phage. Phage lysins are of high interest in this regard as they digest the bacterial cell wall during infection and are less likely to invoke resistance in the host when compared to antibiotics. This study aims to investigate the tail-associated lysins (TALs) in DFU host associated phage and prophage genomes with a particular interest in *S. aureus* and *S. epidermidis*. Phage genomes were obtained from NCBI virus database and examined using RAST webserver. The identification of TALs was carried out using NCBI domain databases and Pfam. PHASTER webserver was used to locate intact prophage present in *S. aureus* and *S. epidermidis* to then identify TALs within prophage genomes. Although the research is still in progress, TALs with domains such as endopeptidase, lytic transglycosylase and amidase have been identified. In the future, selected TALs will be synthesised and expressed *in vitro* to investigate their antimicrobial activity and efficiency. This work will allow for genetic engineering of such endolysins for both detection and control of *Staphylococcal* pathogens.

B036

Understanding how *Streptomyces* control cell division during DNA damage repair

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Abstract

DNA damage often results in a pause of cell division until genome integrity is restored. In bacteria, a widely conserved response to DNA damage is the SOS response which relies on two proteins: the multifunctional recombinase RecA and the transcriptional repressor LexA. Under DNA-damaging conditions, this response activates proteins involved in DNA repair and the inhibition of cell division which in most unicellular bacteria results in temporary filamentous growth. However, it is unknown how naturally filamentous growing bacteria like *Streptomyces* cope with DNA damage and how DNA damage repair is coordinated with cell division.

To identify novel regulators of cell division in *Streptomyces* that specifically function during DNA damaging growth conditions, we investigated the global response of *Streptomyces venezuelae* to several genotoxic agents, including mitomycin C, ciprofloxacin, and methane methylsulfonate. We performed ChIP-seq experiments to identify the LexA regulon in *Streptomyces* and conducted RNA-seq experiments to determine the global transcriptomic response to DNA damaging agents in the wildtype and a D*recA* mutant. The combined analysis of the available data sets provides a comprehensive overview of the SOS response in *Streptomyces* and has facilitated the identification of a potential DNA-damage induced cell division inhibitor. We are currently characterising the function of this novel cell division regulator to understand how the activity of the cell division machinery is affected following induction of the SOS response, using a combination of molecular, biochemical and cell biological approaches.

B037

Characterising the Two-Component System-Regulated Biosynthesis of Formicamycin in Streptomyces formicae

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Abstract

Derivatives of the secondary metabolites of Streptomyces bacteria account for over half of the antibiotics currently used in the clinic. A crucial part of overcoming antimicrobial resistance (AMR) is in the development of new antibiotics that function with a novel mechanism of action, to avoid the rapid acquisition of microbial resistance. We previously reported the isolation of the new species *Streptomyces formicae* from African Tetraponera penzigi plant-ants, shown to have potent activity against several strains on the World Health Organisation's AMR watch list including MRSA. Genetic analysis of this strain revealed one of its 45 biosynthetic gene clusters (BGCs), a type 2 polyketide synthase, produced all 13 formicamycins, the natural products responsible for *S. formicae*'s antimicrobial activity. Several regulators of the formicamycin biosynthetic pathway have been identified, including the major activator, a two-component system (TCS) ForGF. TCSs are present in many secondary metabolite BGCs, however their functions remain largely unknown. Gene-reporter fusion assays have helped identify the promoters under the control of ForGF. The TCS is now being fully investigated using a combination of CRISPR/Cas9 mutagenesis and biophysical characterisation of the purified proteins in order to identify the activating signal of the cytoplasmic sensor kinase and the binding sites of the response regulator. We have shown that manipulating ForGF leads to overproduction of the formicamycins and therefore hypothesise that cluster-situated TCSs in other BGCs could be a target for manipulation for overproduction of other novel antimicrobial molecules for clinical development.

B038

Surface protein display in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a Gram-positive human pathogen responsible for many infections worldwide. Surface proteins, covalently bound to the cell wall, are important virulence factors, and make potential targets for vaccine development, therefore understanding protein secretion is important for the development of future control regimes. While mechanisms of protein secretion through the bacterial membrane have been well characterised, less is known regarding the process of protein translocation across the cell wall. Recent atomic force microscopy of the S. aureus cell wall has revealed surface pores of varying sizes throughout the cell wall, however it is unclear if these aid protein secretion in any way.

Within the N-terminal signal peptide of many covalently bound surface proteins is the presence of a YSIRK-GXXS motif. The YSIRK signal peptide results in the localisation of proteins at the septum during cell wall formation, resulting in the display of the protein on the cell wall at the end of the division cycle.

Bioinformatics analysis performed in preliminary research showed that the YSIRK signal peptide was more common among larger surface proteins (of around >50kDa), suggesting that the motif is perhaps a requirement for larger proteins to be displayed across the cell wall. Using a set of specifically designed protein constructs, protein analysis, and fluorescence microscopy, the aim of this project is to determine the role of YSIRK in protein secretion and better understand how large surface proteins are secreted in S. aureus.

B039

Type II secretion system contributes to fitness maintenance and secretion of many extracellular enzymes in Chromobacterium violaceum

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Abstract

The type II secretion system (T2SS) is a multiprotein device for the secretion of extracellular proteins. *Chromobacterium violaceum,* a Gram-negative beta-proteobacterium, has an operon with 12 genes encoding a potential T2SS. To define the role of the T2SS in *C. violaceum*, we constructed four T2SS null mutant strains, Δ*gspD*, Δ*gspE*, ΔT2SS, and Δ*pilD*, and their respective complemented strains. Growth curves, CFU counting, and live and dead assay revealed that the T2SS mutant strains had reduction of viability. The deletion of the T2SS genes compromised the outer membrane, as demonstrated by growth curves with the antibiotic polymyxin B and permeability assays with ethidium bromide. Transmission electron microscopy showed that T2SS mutant cells had morphological defects. Degrading substrate plate assays were performed in M9 medium supplemented with specific substrates. All mutant strains showed decreased chitinase, gelatinase, hemolysin, and protease activity. Proteolytic activity assay was performed in M9 medium supplemented with skim milk and EDTA. In the presence of EDTA, the proteolytic activity of the wild strain decreased, indicating that the proteases secreted are metalloenzymes. Analysis by SDS-PAGE and quantitative proteomics indicated that the secretomes of the wild type and Δ*gspD* mutant strains were very distinct, revealing many degradative enzymes as T2SS targets. The expression of the T2SS operon increased along the growth curve and in the presence of bile salts. In conclusion, *C. violaceum* has a functional T2SS necessary for the secretion of degradative extracellular enzymes. Disturbing the T2SS machinery compromises the *C. violaceum* fitness even in regular growth conditions.

B041

Of antibiotics and Streptomycetes: bioprospecting isolates from a high-altitude ecosystem in South America

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Abstract

Bioprospecting of underexplored environments and microbiomes is one of the key strategies for drug discovery. Páramos, a high-altitude ecosystem and evolutionary hotspot in the northern Andes of South America, harbour microbial diversity yet to be studied for its potential for antibiotic production.

This work focuses on a set of strains isolated from a páramo in Colombia. Bioassays, phylogenetic analyses, and genome mining suggested the strain CG926 was the most promising one. It was found to have an 8.5 Kb genome, be closely related to *Streptomyces avidinii* and contain around 25 biosynthetic gene clusters (BGC) potentially related to novel compounds.

Interesting clusters from this strain were screened using a cosmid library and a NRPS- T1 PKS was heterologously expressed in *S. coelicolor* M1146. The cluster selected for heterologous expression is a 70 kb BGC that also contains within it a stravidin BGC, *svn*A-N, including two flanking streptavidin genes.

We carried out antimicrobial and mode of action assays and chemical analyses in UHPLC-HRMS to explore the features of this potentially novel bioactive compound. EtOAc extracts of the *S. coelicolor* M1146 transformed with it revealed antimicrobial activity against Gram-Positive and Gram-Negative indicator strains while preliminary data suggested a mode of action related to DNA damage. Overall, these results indicate that the strain CG926 from an underexplored environment harbours the potential for novel antibiotics.

B042

Pathways to promiscuity in evolving bacterial transcription factors are biased by pre-existing gene regulatory network structure

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Abstract

Gene regulatory networks (GRNs) are fundamental control circuits that allow bacteria to vary their metabolism and physiology in response to environmental challenges. A key mechanism of change in GRN structure is gain of promiscuity (or crosstalk) by a component transcription factor. This non-canonical activity allows evolution of new regulatory connections, however the drivers determining how promiscuity emerges within GRNs are largely unknown. Utilising experimental evolution, we investigate the emergence and refinement of promiscuous transcription factor activity in the bacterium *Pseudomonas fluorescens*. In this model, the nitrogen regulatory protein NtrC repeatably becomes promiscuous and rescues flagellar gene expression when an immotile strain lacking the flagellar regulator FleQ is placed under selection to swim. NtrC and FleQ share structural homology, but it is unclear why NtrC is consistently utilised to the exclusion of all other FleQ homologs. Using a Δ*fleQ* Δ*ntrC* double knockout we investigated the evolution of promiscuity in an alternative regulator, allowing identification of commonalities and constraining factors. We found three separate mutational pathways that generate promiscuity, which altered: (i) regulator expression; (ii) global gene regulation; and, (iii) core metabolism. A key commonality linking these pathways was the pre-existing GRN structure around the transcriptional regulator involved. We propose that GRN architecture is key in creating and constraining opportunities for promiscuity and the repurposing of a transcriptional regulator. This grants insight into the viability of a particular regulator in restructuring regulatory networks beyond simple structural homology and has broad implications for the evolution of regulatory structure and function in bacteria.

B043

Functional Recombinant Expression of Human Cytochrome P450s in Escherichia coli

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Abstract

Human cytochrome P450s (CYP) are membrane bound monooxygenases, located in the cytoplasm of the endoplasmic reticulum in liver tissue, and contain haem b as a cofactor. P450s are the major metabolisers of the drugs in the human body. They catalyse the conversion of lipophilic to hydrophilic forms, resulting in the excretion of compounds through the kidneys (Quehl et al., 2016). Therefore, P450s have an important role in clinical pharmacology. During drug oxidation the required electrons are derived from nicotinamide adenine dinucleotide phosphate (NADPH), are delivered by the P450 reductase (CPR) through its cofactors (Munro et al., 2007, Urlacher and Girhard, 2012, Quehl et al., 2016).

Class II Isoforms CYP2A6, CYP2B6, CYP3A4, CYP3A5 and CYP2D6 belong to two of the three families (CYP1, CYP2 and CYP3) that metabolise 75-80% of the drugs in use (Fisher et al., 2009).

The genes encoding the above P450s have been co-cloned, with human reductase, in an *E. coli* host using a linker. The expression level of co-cloned enzymes was compared against the equivalent co-expressed enzymes in two different plasmids. More substantial expression was achieved using a chaperone folding system. Successfully expressed enzymes were used in whole cell biotransformation assays to determine their catalytic activity. Moreover, two candidate metagenome sequences, homologous to CYP3A5 were cloned and expressed recombinantly.

B044

First things first, primary metabolism in *Streptomyces.*

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Abstract

To combat the antimicrobial resistance crisis there is a need to develop new antibiotics and increase the production of existing antimicrobials. Pyruvate kinase (Pyk) has been shown to be a good target for metabolic engineering to increase antibiotic production, catalysing the irreversible conversion of phosphoenolpyruvate (PEP) to pyruvate. Pyruvate phosphate dikinase (PPDK) in contrast catalyses the reverse in a three-step process converting pyruvate, ATP and inorganic phosphate into AMP, PEP and diphosphate. There are two copies of Pyk in the majority of *Streptomyces* and two copies of PpdK, indicating there are four potential biochemical routes for the interconversion of pyruvate to PEP. There are currently no studies involving the role of PPDK in Actinobacteria, however previous work has shown that one of the Pyk enzymes in *S. coelicolor* is upregulated 30-fold prior to antibiotic production on gluconeogenic substrates.

Here we investigate the role of PPDK in *Streptomyces* by creating knockdown mutants using CRISPRi /dCas9 technology and over-expressing of the genes in WT strains. These strains exhibit both growth and developmental defects, suggesting that PpdK plays a key role in central carbon metabolism in *Streptomyces*. We hope this enables us to better understand the role of PPKD in the primary metabolism of streptomycetes and the flow of metabolites around the pyruvate-phosphoenolpyruvate-oxaloacetate node of central metabolism under a range of conditions and to understand how this impacts the availability of precursor molecules for specialised metabolite production.

B045

Commensal gut bacterial genus *Bifidobacterium* shows wide range of inter- and intra-species variation in utilising fermentable fibre resistant starch impacting beneficial metabolite production

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Abstract

Gut microbiota members such as *Bifidobacterium longum* and *B. bifidum* dominantly colonise the infant gut by utilising human breast milk oligosaccharides, aiding immunodevelopment. Post-weaning, *B. adolescentis* and *B. breve* utilise other dietary substrates including resistant starch (RS), being a type of fibre. Bifidobacterial fermentation of RS releases by-products such as maltose and beneficial short-chain fatty acid acetate into the intestinal milieu which benefit the microbial community and host gut health.

The *Bifidobacterium* genus-wide scope of RS fermentation and the biomolecular underpinnings of this starch-bacterium relationship are not fully explored. 11 bacterial isolates from pre-term infant stool and 5 other selected strains were investigated. Using dbCAN2 to detect sequence homology to carbohydrate-active enzymes, results show a wide spectrum in the number of enzyme families associated with starch binding and hydrolysis, which corresponds with starch utilisation growth rates. A strain of *B. pseudolongum* had a reduced growth rate utilising resistant maize starch compared to normal maize starch. Meanwhile, another *B. pseudolongum* strain showed no difference between starch types, indicating intra-species variation in starch utilisation hinging on ability to produce coordinative degradative enzymes. ^{1}H-NMR metabolite analysis of 7 starch-degrading strains (representing 3 species) also showed a marked increase in acetate and maltose during *in vitro* starch fermentation. To conclude, bifidobacteria have varied capacity to pivot to starch fermentation and produce acetate depending crucially on starch structure. Revealing the biometabolic impacts of the RS-bifidobacteria relationship could contribute to personalised nutrition approaches or synbiotic development, targeted to improve infant and adult health.

B046

The role of microbiome-derived metabolites in tumour regression.

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Abstract

Cancer causes a death of large number of people globally every year, with limited treatment options available including chemo-, immuno-, hormonal-, and surgical therapy, each with their own unpleasant side effects. Given recent studies highlighting a potential role for the gut microbiome in influencing outcomes in cancer and it's treatment, here we examined the effect of two microbiome-derived metabolites produced by Lachnospiraceae called 4-(trimethylammonio) pentanoate (4-TMAP), and 3-methyl-4-(trimethylammonio) butanoate (3M-4-TMAB). These metabolites inhibit fatty acid oxidation and induce mitochondrial dysfunction in murine liver and brain cells and here we determined whether they would exert effects on tumour metabolism and growth in three different breast, prostate and melanoma cancer cell lines and 3D culture spheroids; BT549, PC-3 (which is known as highly depends on fatty acid oxidation for survival and progression), and B16F10. In addition, we examined effects of 3M-4-TMAB and 4-TMAP in combination with approved chemotherapeutics for breast and prostate cancer (docetaxel and MCL-1 inhibitor). Changes in cancer cell viability and fatty acid metabolism were noted in bacterial metabolite treated cells but no change in ATP or triglyceride levels. Additional no additive decrease in cell viability was noted when used in combination with chemotherapeutics. However, this data indicates that circulating microbiome-derived metabolites can exert significant metabolic effects on cancer cells.

B047

A comprehensive screening of the two-component network in *Staphylococcus aureus*

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Abstract

The pathogenesis of *Staphylococcus aureus* (*S. aureus*) involves a complex interplay between the host environment and expression of virulence factors, such as cytolytic toxins. In this bacterium, the primary method of sensing environmental signals and adapting gene expression are two-component systems (TCS), which combine signal transduction into a membrane-bound histidine kinase (HK) and a cytosolic response regulator (RR). In *S. aureus*, several TCSs are well defined as core regulators of cytolytic activity, but a comprehensive understanding of this relationship in the entire network has eluded us as previous studies have been conducted in a wide range of strains and growth conditions. To overcome this, we screened cytolytic activity in a collection of transposon mutants of each TCS in the genome of *S. aureus* JE2. This identified several TCSs mutants with reduced cytolytic activity including the phoP mutant, a mutant in the RR of the phosphate sensing TCS, PhoRP. Further investigation of the phosphate system in S. aureus identified PitR, a regulatory protein in an operon with the PitA phosphate transporter, as a repressor of cytotoxicity. Moreover, we identified that the phoP mutant has higher cytotoxicity than JE2 under phosphate limiting conditions, suggesting PhoRP signalling represses cytotoxicity in these conditions. We hypothesis that this response is dependent on PhoRP sensing phosphate limitation and inducing expression of the pit operon, including PitR. Overall, this project uncovers a previously unknown link between phosphate homeostasis and cytotoxicity and establishes phosphate as an important environmental signal for coordinating the pathogenicity of *S. aureus*

B048

Glutamate Dehydrogenase (GdhA) of *Streptococcus pneumoniae* is required for high temperature adaptation

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Abstract

During its progression from the nasopharynx to other sterile and nonsterile niches of its human host, Streptococcus pneumoniae must cope with changes in temperature. We hypothesized that the temperature adaptation is an important facet of pneumococcal survival in the host. Here, we evaluated the effect of temperature on pneumococcus and studied the role of glutamate dehydrogenase (GdhA) in thermal adaptation associated with virulence and survival. Microarray analysis revealed a significant transcriptional response to changes in temperature, affecting the expression of 252 genes in total at 34°C and 40°C relative to at 37°C. One of the differentially regulated genes was gdhA, which is upregulated at 40°C and downregulated at 34°C relative to 37°C. Deletion of gdhA attenuated the growth, cell size, biofilm formation, pH survival, and biosynthesis of proteins associated with virulence in a temperature-dependent manner. Moreover, deletion of gdhA stimulated formate production irrespective of temperature fluctuation. Finally, Δ*gdhA* grown at 40°C was less virulent than other temperatures or the wild type at the same temperature in a *Galleria mellonella* infection model, suggesting that GdhA is required for pneumococcal virulence at elevated temperature.

B049

Enhancing an artificial urine for culture and *in vitro* studies of uropathogenic *Escherichia coli.*

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Abstract

Uropathogenic *Escherichia coli* (UPEC) are the most common cause of urinary tract infections, which pose a great burden on global health and the economy through morbidity, mortality and loss of productivity. Pooled human urine can be used as a growth medium for *in vitro* studies, however, composition can vary depending on a number of factors, even if the same donors are used. There have been a number of artificial urine formulas made as an alternative to pooled human urine. However, these artificial urines do not always replicate the growth rates of UPEC strains in pooled human urine and may contain components not always present in healthy human urine. We have used growth studies to show that a multipurpose artificial urine does not support growth of UPEC strain CFT073. We have shown, using liquid chromatography mass spectrometry (LC-MS) and RNA-seq, that the multipurpose artificial urine has a different metabolome and induces a distinct transcriptome in CFT073 compared to pooled human urine. We have shown that the addition of amino acids and other metabolites identified by LC-MS enhances bacterial growth and induces a more similar transcriptome than the multipurpose artificial urine. However, the pattern of gene expression remains distinct from that induced by pooled human urine. Further work to modify the enhanced artificial urine could produce a reliable and reproducible alternative to pooled human urine for *in vitro* studies of UPEC physiology, metabolism, and gene expression.

B050

EnvR is a potent repressor of acrAB expression in *Salmonella* Typhimurium

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Abstract

Resistance-nodulation-division (RND) efflux pumps confer multi-drug resistance (MDR) in Gram-negative bacteria by facilitating the complete extrusion of clinically relevant antibiotics. From the TetR family of transcriptional regulators (TFTRs), EnvR has previously been shown to act as a repressor of *acrAB* transcription, the primary RND pump in *E. coli*. We have now determined the precise binding site of EnvR upstream of the *acrAB* operon in *Salmonella enterica* serovar Typhimurium SL1344 and shown that EnvR is able to potently repress expression of the *acrAB* transcript and AcrAB protein. Over-expression of EnvR in *S.* Typhimurium SL1344 significantly increased intracellular accumulation of Hoechst H33342 compared to wild-type SL1344 and increased susceptibility to antimicrobials on a substrate-dependent basis. Over-expression of EnvR also conferred other phenotypic changes associated with lack of efflux including reduced bacterial motility and an inability to infect human intestinal cells *in vitro*. Taken together, this work indicates that EnvR can prevent expression of AcrAB, rendering the bacteria avirulent and susceptible to antibiotics. Exploiting the functions of regulators like EnvR thus appears to be a promising avenue to combat antibiotic resistance.

B051

SYMBIOSIS BETWEEN MEMBERS OF THE MICROBIOTA TO DEGRADE COMPLEX POLYSACCHARIDES FROM ALTERNATIVE FOOD SOURCES

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Abstract

The human gut microbiota (HGM) contributes to the physiology and health of its host in a variety of ways, maintaining gut homeostasis and modulating the immune system but also allowing the use of a wide range of alternative meat substrates, especially polysaccharides from new foods with less environmental impact, such as fungal mycoprotein. The health benefits provided by dietary manipulation of the HGM require knowledge of how these polysaccharides may shape the gut microbiota community, specially β-glucans which have been described as potential prebiotics. Nevertheless, the mechanisms of action underpinning these health effects related to β-glucans are still unclear, and its precise impact on the complex gut microbiota community has been subject to debate and revision.

The utilization of a mixed linkage fungal β-glucan as energy source by gut bacteria species have not been studied so far, and it will clarify the different effects that these polysaccharides may have on the populations of gut bacteria and the health of the host, showing us its potential as a new prebiotic and its power as a realistic alternative for the excessive meat consumption in the current fat-based diet.

This project explored how different *Bacteroides* and *Bifidobacterium* spp. can use β-glucan from the fungus *Fusarium venenatum,* which is used to elaborate mycoprotein. The study was focused on the search of the metabolic pathways for the β-glucan degradation together with the presumable cross-feeding relations established between different members of the HGM involved in the process.

B052

Elucidating the mechanism of a bacterial NAD^+-depleting RES-domain toxin

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Abstract

Widespread use of antibiotics has led to multidrug-resistant bacterial pathogens and detailed knowledge of bacterial survival and defense mechanisms is urgently needed. Bacterial toxin-antitoxin (TA) systems are important cellular regulators, encoding a toxin that inhibits cell growth and an antitoxin that neutralizes the toxin. We recently identified a novel TA system, Xre-RES, in *Photorhabdus luminescens*. The RES-domain is named after three conserved amino acids; arginine (R), glutamate (E) and serine (S), predicted to support a metal-independent RNase activity. We showed that Xre-RES functions as a bona-fide TA system in *Escherichia coli* and that the RES-domain toxin efficiently inhibits transcription and translation upon expression. Moreover, we have solved a 2.2 Å crystal structure of the intact RES^{R23A}-Xre TA complex from *Pseudomonas putida*, revealing an unusual 2:4 stoichiometry. Surprisingly, RES showed structural similarity to ADP-ribosyl transferases (ARTs), yet, an atypical binding pocket suggested that the toxin functions as a NADase. Supportive of this, we showed that toxin expression led to NAD^+ depletion in *E. coli*. Using the structure, we predicted residues likely to be involved in NAD^+ binding and/or degradation and we showed that amino acid substitutions of these residues neutralizes toxicity of *P. putida* RES. Finally, we have solved a 1.55 Å structure of the *P. putida* RES^{R5A} toxin. This is, to our knowledge, the first structure of free RES-domain toxin, enabling comparison between active and bound toxin. We are currently working on purifying and crystallizing free RES with NAD^+ to further increase our understanding of the RES-domain toxin.

B053

The matrix reloaded: biophysical and biochemical analysis of extracellular matrix components in *Pseudomonas aeruginosa* biofilms

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Abstract

Pseudomonas aeruginosa (PA) is an opportunistic human pathogen that is responsible for high morbidity and mortality in individuals who are predisposed towards infection, such as the immunocompromised or people with cystic fibrosis (CF). In people with CF, PA often causes chronic infections in the airways – infections that are now known to be associated with biofilm formation. However, we still understand remarkably little about the sessile lifestyle. What we do know is that the biofilm matrix – the "glue" that holds the biofilm cells together - confers tolerance to numerous external stresses such as antibiotics and immune factors, and also provides physical resistance to shear stress.

We now know that proteins, polysaccharides, lipids and nucleic acids are all found in the PA biofilm matrix, and that each component can play an important role in holding the biofilm together. However, the precise contribution of each matrix component to the overall mechanical stability of the biofilm remains unclear. To investigate this further, we have made defined deletions in each of the biofilm-associated genes: *pslD*, c*drA*, *sadB* and *cupA3*. Using these, we have been investigating how each encoded gene product contributes towards the "stickiness" of the biofilm matrix. This is being done by measuring the force required to detach individual cells from the mutant biofilms using a state-of-the-art optical tweezers setup. In parallel, we have been examining how each of these genes contributes towards defining the "matrixome" of the biofilm (i.e., that complement of secreted proteins that are spatially localised in the matrix compartment).

B056

Aurodox: Repurposing Old Drugs for Bad Bugs

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Abstract

Aurodox, an Elfamycin antibiotic produced by *Streptomyces goldiniensis*, exhibits antibiotic activity against Gram-positive bacteria, by inhibiting translation via elongation factor EF-Tu. A novel mode of action was discovered for aurodox, where inhibition of Type III Secretion Systems of Enteropathogenic (EPEC) and Enterohemorrhagic (EHEC) *Escherichia coli* was shown to be an effective antivirulience treatment. The *S. goldiniensis* genome was sequenced and putative aurodox biosynthetic gene cluster (BGC) identified. Cloning and heterologous expression of the putative aurodox BGC (AurI) in *Streptomyces coelicolor* M1152 confirmed its responsibility for aurodox biosynthesis. Computational analysis of the BGC revealed a multimodular polyketide synthase pathway similar to the BGC of kirromycin. We hypothesised that the final step of aurodox biosynthesis was converting kirromycin, to aurodox via a SAM-dependent *O*-methyltransferase on the pyridone moeity, catalysed by the BGC encoded AurM*. Cloning and expressing AurM* in the kirromycin producer, *S. collinus*, resulted in aurodox production. Aurodox resistance genes at distinct genetic loci were also identified during sequencing. A Major-Facilitator Superfamily protein (AurT), present within the aurodox BGC, was thought to confer self-resistance to the producer, through aurodox efflux. However, heterologous expression indicated that *aurT* alone was insufficient for resistance. An additional, Elfamycin-resistant, copy of EF-Tu (*tuf2*) was identified in the genome. Cloning *tuf2* and expression in *S. coelicolor* M1152_AurI indicated that both *tuf2* and *aurT* are required for aurodox immunity in the producing strain. Elucidation of a*urM**, *aurT* and *tuf2*'s role has allowed dissection of aurodox biosynthesis. Further genetic manipulation of the BGC could lead to production of novel derivatives.

B057

Investigation into the effects of fatty acids in the diet of swine on immune response and the gut microbiome.

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Abstract

Previously, in-feed antibiotics were used to enhance performance gains and counteract adverse effects of weaning. However; an EU-wide ban prohibiting the use of antibiotic growth promoters was issued in 2006, prompting the search for safe and innovative alternatives. Studies have indicated that dietary fatty acids can minimise weaning disorders including elevated incidence of enteric disease and immunodepression. Furthermore, research has indicated that including fatty acids in the porcine diet has a positive effect on performance; increasing growth rate and enhancing feed conversion. It is thought these health and production benefits come from an immunomodulatory role of the fatty acids, however; the mechanisms through which these changes occur are not well understood.

The aim of this research was to collaborate with local feed industry in order to produce relevant information regarding the use of fatty acids as a swine health supplement and alternative to growth-promoting antibiotics. To do this, a combination of in vitro assays and field studies were used to investigate the immunomodulatory and anti-viral properties of a unique blend of fatty acids. Additionally, deep sequencing and metagenomic methods were employed to explicitly explore the influence fatty acid supplementation has on shaping the intestinal microbial community during the weaning period. Results indicate that fatty acids act to initiate and enhance immune response to infection and also play a role in modifying the intestinal microflora, establishing an environment that favours the growth of commensal species such as *Lactobacillus*. The fatty acid blend also demonstrated anti-viral activity against porcine rotavirus *in vitro*.

B058

The TbD1 locus mediates a hypoxia-induced copper response in *Mycobacterium bovis*

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Abstract

Mycobacterium tuberculosis and *Mycobacterium bovis,* cause human tuberculosis and bovine tuberculosis, respectively, causing huge threats to public health and considerable economic losses to the agricultural industry. Although *M. tuberculosis* and *M. bovis* share over 99.9% genome identity, they show distinct host adaptation; hence, while the molecular basis of host adaptation is encoded in their genomes, the mechanistic basis is still unclear. Exploration of the *in vitro* phenotypic consequences of known genetic difference between *M. bovis* and *M. tuberculosis* offers one route to explore genotype-phenotype links that may play a role in host adaptation. The TbD1 ('*Mycobacterium tuberculosis* deletion 1 region') is absent in *M. tuberculosis* 'modern' lineages but present in 'ancestral' *M. tuberculosis*, *M. africanum* and animal adapted strains, such as *M. bovis*. The function of TbD1 has previously been investigated in *M. tuberculosis*, where conflicting data has emerged on the role of TbD1 in sensitivity to oxidative stress, while the underlying mechanistic basis is unclear. We aimed to shed further light on the role of the TbD1 locus by exploring its function in *M. bovis*. *Mycobacterium bovis* ΔTbD1 mutants were constructed and comparative transcriptomics was used to define global gene expression profiles of *M. bovis* WT and the ΔTbD1 strains under *in vitro* culture conditions. This revealed differential induction of a hypoxia-driven copper response in WT and ΔTbD1 strains. *In vitro* phenotypic assays demonstrated that the deletion of TbD1 sensitized *M. bovis* to H_2O_2 and hypoxia-specific copper toxicity. Our study provides new information on the function of the TbD1 locus in *M. bovis* and its role in stress responses in the MTBC.

B059

LHR, a protein found across all domains of life, begins to reveal its functions.

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Abstract

Changes to DNA structure frequently inhibit essential processes such as DNA replication. Overcoming replication blockage or collapse requires replication-coupled DNA repair enzymes that catalyse removal of aberrant DNA structures and chemically modified bases. The LHR family of helicases that consist of 'Lhr-core' and 'Lhr-extended' forms are present throughout archaea and several bacterial clades. The functions of Lhr in specific DNA repair processes is beginning to be revealed, and we will report our latest findings from analyses of archaeal and bacterial Lhr proteins.

Our previous work characterised recruitment and activities of archaeal Lhr, an Lhr-core enzyme, on model replication forks substrates (Buckley *et al* Biochem. J., 2020). We will report new data about the 'Lhr-extended' enzyme from *E. coli*— this includes (a) a newly identified DNA glycosylase activity *in vitro* and (b) genetic analysis implicating Lhr in a novel mutation repair pathway, and in overcoming oxidative stress through interaction with a Rad51 paralogue. The data we present is part of an emerging story about the contribution of Lhr and its associated proteins in prokaryotic DNA repair, which may extrapolate into roles for it's human homologues that are of currently unclear function.

B060

Chronic and Recurrent Bacterial Infections: The Role of Persisters and Biofilms

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Abstract

Bacterial chronic and recurrent infections are difficult to treat due partially to the existence of biofilms and antibiotic persister cells. Bacterial biofilm is an accumulation of bacterial cells organized into a coordinated functional community. The dispersal of bacteria and adaptation to available nutritional and environmental conditions contribute to the development of biofilm. In the human host, bacteria can form biofilm on both living (biotic) and non-living (abiotic) surfaces. The interaction between bacterial cells in bacterial biofilm can cause chronic and recurrent infections by forming a physical barrier to protect the microbe from host immune system and antibiotics treatment. Persisters are a subpopulation of genetically sensitive bacterial cells that survive high concentrations of antibiotics. Whilst resistant bacterial cells have stable and inheritable drug insensitivity, persisters survive antibiotic treatments by entering a dormant or semi-dormant state and resume growth when antibiotic pressure drops. Survival advantage of bacterial persisters has been observed among different classes of antibiotics, including beta-lactams and fluoroquinolones. Persister cells in biofilms are responsible for the recalcitrance of chronic and recurrent bacterial infections since persisters remain viable and promote the accumulation of bacteria to form biofilms when the level of antibiotics drops. Persisters and biofilms are both associated with the bacterial survival mechanisms responsible for chronic and recurrent infections but the role of each mode of survival remains unclear. We explored the literature and asked whether biofilms, persisters or both are required for the establishment of chronic and recurrent infections, aiming to a more complete understanding of this topic.

B061

Investigating inter-protein relations of the GAC biosynthesis machinery

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Abstract

Group A *Streptococcus* (GAS) elicits a wide range of infections, which can develop into the lethal autoimmune post-streptococcal disorders, responsible for the majority of GAS related deaths. The essential Group A carbohydrate (GAC), a rhamnose containing polysaccharide displayed on the cell surface, has been implicated in the ability of GAS to evade the host immune system and therefore identified as a novel virulence factor. The GAC biosynthesis proteins B-G (GacB-G) are conserved in many streptococci and are responsible for the production of the polyrhamnose backbone. It is not known if GacB-G interact with one another or are regulated, spatially and temporally within the cellular context. Here we show that GacB-G, when expressed in the model organism *E. coli*, localise to the membrane and strongly associate with each other in various protein-protein interaction studies. With the use of a bacterial Two-hybrid assay we found that N-terminally tagged GacB, C and E situate close to N-terminally tagged GacB, C, E, F and G. Furthermore, the studies reveal the possibility of multimer formation for GacB, GacC and GacE. Investigation of the GC biosynthesis proteins in a pull-down assay revealed that both GacE^{FLAG} and ^{HA}GacC form stable interactions with GacB-G. This work begins probing the relationship of GAC biosynthesis proteins (B-G), with a clear revelation that GacB-G forms a multi-protein complex. Understanding the PPI between GAC biosynthesis proteins could reveal potential drug targets, that could reduce future GAS related infections and post-streptococcal diseases.

B062

Characterising the role of TssA proteins in the assembly and dynamics of the type VI secretion system

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Abstract

The gram-negative, opportunistic pathogen *Pseudomonas aeruginosa* encodes many weapons for virulence and interbacterial warfare, including the type VI secretion system (T6SS), a contractile nanomachine delivering toxins into eukaryotic and prokaryotic cells. The T6SS component TssA is required for T6SS activity and is central to the assembly of the T6SS contractile sheath. Despite this requirement, TssA proteins vary in size, structure and oligomeric state, with T6SS clusters encoding either a long or short TssA protein. Using the three *P. aeruginosa* T6SSs, I am investigating the structural and functional differences between these TssA forms, and how they may contribute to differences in T6SS dynamics. The role of TssA proteins in T6SS activity can also be understood by dissecting their interactions with other T6SS proteins, including identifying which sub-domains are required for these interactions. Imaging of real-time protein dynamics through fluorescent microscopy allow the dynamics and interactions of T6SS components to be visualised, to understand the contribution of TssA to T6SS function. These data contribute to our understanding of the role of TssA, its interactions, association with accessory proteins and how these characteristics might influence physiologically relevant T6SS activities.

B063

Ammonium transport and fungal pathogenicity

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Abstract

Cellular ammonium transport is facilitated by the ubiquitous Amt-Mep-Rh superfamily; members of which have been identified in every branch of the tree of life. The physiological relevance of Amt-Mep-Rh proteins extends beyond their role in ammonium acquisition as a nitrogen source. In fungi for instance, in the presence of very low ammonium concentrations, specific Amt-Mep-Rh transporters, the Mep2-like proteins, have been proposed to act as sensors required for the development of filamentous growth, a dimorphic switch associated to the virulence of pathogenic fungi. Two highly conserved histidine residues protrude into the lumen of the pore of these transporters, forming the family's characteristic Twin-His motif. Here, using a combination of in vitro electrophysiology, in silico molecular dynamics simulations and in vivo yeast functional complementation assays, we demonstrate that variations in the Twin-His motif trigger a mechanistic switch between a specific transporter to an unspecific ion channel activity. We show that coexistence of both mechanisms in single Twin-His variants of yeast Mep2 transceptors disrupts the signalling function and so impairs fungal filamentation. Filamentation is often related to the virulence of pathogenic fungi, such as the human pathogens Candida albicans, Histoplasma capsulatum or Cryptococcus neoformans. In this context, our results are of particular importance as the characterisation of the conditions regulating the yeast dimorphism may be crucial to better understand fungal virulence.

B064

Adaptation delay causes a burst of mutation in bacteria responding to oxidative stress

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Abstract

The relative timing of phenotypic and genetic changes is critical for the adaptation of bacteria to stress conditions. Rapid phenotypic responses allow cells to survive transient stresses whereas population survival during severe stress relies on evolutionary rescue via the emergence of adaptive mutations. These mutations may be present as standing genetic variation or generated de-novo during stress. Here, we explored how mutation rates change in real-time when bacteria become stressed and how these dynamics relate to the underlying molecular mechanisms of adaptation. We employed a range of single-molecule and single-cell microscopy assays to image adaptation of *Escherichia coli* to oxidative stress during continuous hydrogen peroxide treatment. Using a fluorescent version of MutL, a mismatch repair protein, as a proxy for DNA mutagenesis, we observed that sudden exposure to H_2O_2 causes a transient burst in mutagenesis. We show that oxidative stress signalling by the transcription factor OxyR is fast. However, the short time delay before H_2O_2 scavenging enzymes are induced leaves cells vulnerable to the formation of DNA-damaging hydroxyl radicals via the Fenton reaction. During this delay, the activity of DNA Base Excision Repair enzymes increases but is insufficient to prevent conversion of mutagenic lesions into DNA mismatches. The mutagenesis burst was absent in mutant strains with a constitutive OxyR response or when the stress level increased gradually. Similar observations for cells experiencing alkylation stress show that mutagenesis bursts are a general phenomenon associated with adaptation delays and may have a beneficial role in accelerating mutation supply to facilitate evolutionary rescue.

B065

Effectiveness of drug combination on persister cells and bulk population cells in *Mycobacterium tuberculosis*

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Abstract

Treatment of TB requires combination of drugs that may interact synergistically both positively and antagonistically. Previous research into drug synergy in TB has examined only the bulk bacterial population. However, a sub-population of persister cells exists in all bacterial populations that can survive prolonged exposure to antibiotics and contribute to treatment failure. Persister cells are likely to respond differently to synergistic drug combinations. This study investigates drug synergy specifically in the persister population to identify novel strategies to optimize synergy to kill persister cells more effectively.

To test the efficiency of antibiotic combination on both susceptible cells and persister cells, the checkerboard minimum inhibitory concentration (MIC) assay and time-kill assay were adapted in this study. Rifampicin (RIF) or isoniazid (INH) were combined with either bedaquiline (BDQ) or clarithromycin (CLAR) at various concentrations to test the inhibition effect on *Mycobacterium tuberculosis* (Mtb). The combination of INH with CLAR or both RIF and INH with BDQ were found to effectively inhibit the growth of mycobacterial cells at sub-inhibitory concentrations (1/4* MIC). A significant reduction of persister cells formation was only found with the combination of sub-inhibitory concentrations of RIF and BDQ or INH and CLAR at day 20.

We found that drug combinations are not equally effective on persister cells compared to the bulk population of Mtb. Clearly, antibiotic combinations have to be optimized for both the bulk population and persister cell population of Mtb.

B066

The development of a high-resolution melt curve toolkit to identify lineage defining SARS-CoV-2 mutations in resource limited settings.

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Abstract

Nations with robust epidemiological surveillance systems and next generation sequencing (NGS) capacity have tracked the emergence of severe acute respiratory syndrome-2 (SARS-CoV-2) variants in near real-time, however, many lower middle income countries (LMICs) lack equivalent infrastructure and resources, and as a result, there remains a gap in molecular epidemiology data which is needed to fully explicate the transmission dynamics and emergence of SARS-CoV-2 variants, both within local communities and globally.

To facilitate molecular surveillance of SARS-CoV-2 variants in lower resource settings, alternative methods are desperately needed. The use of RT-qPCR with high-resolution melt (HRM) analysis offers an efficient way to combine SARS-CoV-2 detection with mutation screening, whilst keeping costs and infrastructure requirements to a minimum.

We have designed seven primer sets, validated as three multiplex HRM assays, to enable the detection of lineage-defining mutations present within the SARS-CoV-2 variants of concern: Alpha, Beta, Delta and Omicron. Evaluation of these primer sets and assays using clinical samples with a Ct < 30 and with known complete genome sequence, showed that specificity ranged from 94.2% - 100% and sensitivity was 100% across all primer sets.

Whilst not a replacement for NGS, these multiplex HRM assays help expand global access to molecular surveillance, and further can complement and streamline use of NGS. Further, this agile technology can operate as a molecular surveillance toolkit, which is easily adapted as new variants arise.

B069

GC-MS profiling of Microbial Volatile Organic Compounds released by the pathogenic fungus *Batrachochytrium dendrobatidis*

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Abstract

As the causative agent of Chytridiomycosis, the fungus *Batrachochytrium dendrobatidis* (Chytrid) poses a severe threat to Global frog biodiversity. Ultimately fatal, through invasion and destruction of the skin, chytrid is responsible for the decline of over 500 amphibian species and poses a major risk to both wild and ex-situ frog populations. There is a clear need to develop fast, sensitive methods of chytrid detection to enable the reduction of disease spread and requirement for treatment. Fungi produce Microbial Volatile Organic Compounds (MVOCs): odorous compounds with low molecular masses that are formed by the primary or secondary metabolisms. The composition of these MVOCS profiles which are often specific to individual species of fungi, their growth conditions and other environmental factors meaning chytrid likely produces its own unique volatile fingerprint. Using Solid Phase Microextraction (SPME) and GC-MS, we developed a method for the screening of Chytrid MVOCs released during a variety of environmental conditions. We tested several Chytrid genetic lineages (Bd-GPL, Bd-Brazil and Bd-CAPE) on a variety of liquid/solid media and modified environmental conditions to investigate how these factors influence volatile composition. Our studies revealed specific volatile profiles produced by Chytrid under such conditions. The knowledge of this profile may help inform the development of non-invasive electrochemical sensors used to detect the fungus before clinical manifestation, improving the outlook for *ex-situ* frog conservation.

B070

How to evaluate an assay in the absence of an appropriate gold standard: SARS-CoV-2 molecular testing as a case study

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Abstract

Many assay evaluations for sensitivity/specificity are conducted against a gold standard – indeed, guidelines for such evaluations often implicitly or explicitly assume this is what will be done. The COVID-19 pandemic highlighted an issue that occurs for any truly novel assay: the complete absence of a gold standard. It also highlighted issues that we see commonly with molecular assays: (1) when the new assay has better performance characteristics than the gold standard, the resulting estimates of sensitivity and specificity will be misleading, and (2) when the assay has very high specificity, manufacturer validations in low-throughput laboratories with few samples give little information about real-world performance. With reasonable assumptions about assay properties, it is, however, possible to obtain measures of assay performance by comparing an assay against itself (retesting samples). We shall review the problems of using traditional gold standard approaches in this new world, and shall show how to get useful sensitivity/specificity estimates in a way that avoids the pitfalls of discrepant analysis (a common, but biased, approach to this problem), using a simple maximum likelihood approach, which we applied to our laboratory's SARS-CoV-2 testing. In this way, we shall show how to move easily from reporting molecular assay performance in a narrow way sufficient for regulatory approval but giving inadequate information on real-world utility, to reports that give more information for purchasers deciding whether a product will fit their requirements and end users needing to interpret its output.

B071

Bacterial signals captured from aerosols vary between different sampling matrices and different identities inform development of face mask sampling

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Abstract

Facemasks carrying a sampling matrix (SM) have been used to sample exhalations from TB patients and others[1,2]. The aim here was to investigate the capture efficiency of different SMs with respect to different microbial targets.

Inocula of common bacteria were prepared from mid-exponential broth. Twenty different sampling materials were adjusted into a nebulisation system. OMRON NE-U780 nebulizer was loaded and run for 15 min with air-flow volume and nebulization volume level-II. SKC air pump (15 L/min) adjusted by SKC rotameter was connected to SKC BioSampler. DNA was quantified with 16SrDNA-directed qPCRs with different phylogenetic specificities.

Pseudomonas aeruginosa was most readily captured with the highest proportion of nebulised material captured on polyvinyl alcohol SM, followed in order by *Staphylococcus aureus*, *Moraxella catarrhalis, Mycobacterium bovis* BCG, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Micrococcus luteus* and *Fusobacterium nucleatum*; initial densities of all microbes were ~10^6/ml for nebilisation and the capture efficiency varied over 3 orders of magnitude.

Different SMs were also shown to have different capture efficiencies, again varying over 3 orders of magnitude.

Both the sampling matrix and the microbial target identity appear to be factors affecting capture of microbes from aerosols in face masks. Understanding these factors with be important in further development of face mask sampling.

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B072

Assessment of host DNA depletion methods for rapid molecular detection of bloodstream infection and antimicrobial resistance

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Abstract

Sepsis is a major cause of morbidity and mortality worldwide and existing diagnostics tools are insufficient. Nanopore-based metagenomics can rapidly identify pathogens and antimicrobial resistance determinants, but low bacterial load during bloodstream infection (BSI) compared with the quantity of host DNA from blood makes this approach challenging for sepsis diagnosis. Consequently, we assessed five host depletion strategies that could facilitate such diagnostic approaches.

ATCC strains of Escherichia coli (EC), Pseudomonas aeruginosa (PA), and Staphylococcus aureus (SA) were selected for spiking. Sterile sheep blood was used as host matrix. The ratio of host/pathogen DNA was quantified using primers/probes targeting 16S and 18S rRNA genes. Two commercial [Zymo-HostZERO (C1), MolYsis-Complete5 (C2)] and three published methods described by Charalampous-2019 (P1), Marotz-2018 (P2), and Trung-2016 (P3) were tested.

C1 removed 99.998% (-14.00ΔCq [quantification-cycles];~1.854E+15bp [base-pairs]) and P1 99.978% (-11.00ΔCq;~3.932E+15bp) host DNA. Protocols C2, P3, P2 reduced host by 98.192% (-5.23ΔCq), 87.010% (-2.66ΔCq) and 72.020% (-1.66ΔCq) respectively.

Protocol C1 gained 38.19% (+0.69ΔCq) SA DNA but lost 42.76% (-0.80ΔCq) EC and 41.55% (-0.77ΔCq) PA. P1 led to a higher yield of EC (66.00%;+1.54ΔCq) but 42.76% (-0.80ΔCq) SA and 71.10% (-1.70ΔCq) PA was lost. C2 removed 99.91% (-10.03ΔCq) PA. Bacterial DNA loss was lower/undetected for the other protocols.

While 2 of 5 protocols depleted >99.9% of host DNA, most of them also resulted in moderate to high bacterial loss. Considering <100 CFU/mL in BSI and variable efficiency of DNA extraction kits, it seems important to modify or compensate to improve their sensitivity.

B073

Towards the development of a hand-held RT-LAMP point-of-need assay for foot-and-mouth disease virus

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Abstract

The current gold-standard molecular diagnostic assay for foot-and-mouth disease virus (FMDV) is real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Whilst reliable and accurate, RT-PCR is expensive to establish and run on a routine basis. In contrast, reverse-transcriptase loop-mediated amplification (RT-LAMP) is an isothermal amplification system which, in combination with a lateral flow device (LFD) read-out, can form the basis of an inexpensive, easy-to-use point of need diagnostic system. The objectives of this project were to evaluate RT-LAMP assays for the detection of FMDV RNA using a selection of different reagents, and to explore the use of crude clinical material without prior RNA extraction. We assessed two published LAMP assays against a panel of FMDV isolates (n=79) covering 18 lineages in combination with selected RT-LAMP mastermixes (n=6). Both assays demonstrated broad sensitivity (96.2% relative to RT-PCR) when using the new generation RT-LAMP mix (004-RT) from Optigene. Next, we confirmed the reported capability of LAMP to withstand contaminants which would ordinarily inhibit RT-PCR. The reaction was tested successfully by directly adding dilutions of FMDV-positive epithelial suspension (ES) to the reaction to obtain positive RT-LAMP results. In addition to ES, we tested FLOQSwabs. Oral swabs were collected from cattle experimentally infected with FMDV and incubated in RNAse free water. Testing the water fraction directly generated a positive RT-LAMP signal; RT-PCR testing of RNA extracted from the water confirmed the specificity of this result. The assay is now being on-boarded to a novel, closed platform resulting in a format suitable for use in decentralised locations

B075

Transcriptome analysis of marmosets acutely infected with Zika virus (PRVABC59)

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Abstract

Zika Virus is a member of the *Flaviviridae* which emerged as a significant threat to human health in 2016 following rapid spread globally and in particular throughout South America by transmission from *Aedes* mosquitos. Although the threat posed has substantially receded, understanding virus-host interactions that may impact on host susceptibility remains a topic of interest.

Non-human primates have been developed as models for Zika infection, including both Old and New World species. We previously characterised the dynamics in infection in New World marmosets and tamarins which are both highly susceptible to infection establishing a rapid acute and persisting disseminated infection. Here we explore the early interactions in blood and key lymphoid tissues of common marmosets (*Callithrix jacchus*) challenged with the PRVABC59 Zika strain using RNAseq technology to determine the transcription profiles of these tissues, pairing with uninfected control materials. From mapped reads, using GO enrichment analysis we identified pathways perturbed by infection with this virus, particularly early innate responses and the interferon pathway. Assembling a list of upregulated and downregulated genes and coupled with targeted PCR-based analysis of gene transcription we have investigated the primary response to infection in these susceptible, fully immunocompetent hosts.

These data will inform understanding of mechanisms of early cell signalling in complex infected tissues and factors which may lead to a more persisting infection governed by an acute-phase response.

B076

Evaluation of antiviral activity of Berberine against Corona virus (HCoV-229E) and/or H1N1 influenza virus

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Abstract

In this project, an experimental platform for investigating the antiviral activities of test material was built up in the virology lab of Hamdi Mango Center / the University of Jordan. H1N1 virus was obtained from ATCC and propagated by infecting the virus of (MDCK) host cells. The viral titer was evaluated, and it was about 16 using hemagglutination assay. The effect of berberine on the viral cytotoxicity was evaluated. It found that berberine could reduce the cytotoxicity of H1N1 virus. The mechanism of antiviral activity also was investigated using RT-PCR, it seems that berberine could inhibit the entry of the virus to the host cell. However further future studies are required to confirm these results.

B077

Functional characterization of the HIV-regulating protein, CHD1L, showing Africa-specific human genetic variation

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Abstract

Human Immunodeficiency Virus-1 (HIV-1) remains a global health crisis, highlighting the need to identify new therapies. Given the marked human genome diversity in Africa, we assessed the genetic determinants of control of HIV-1 setpoint viral load (spVL) in 3,879 individuals of African ancestry. We identify a novel association signal on chromosome 1 where the peak variant associates with a ~0.3 log10 copies/ml lower spVL and is specific to populations of African descent. The top associated variant is intergenic and lies between a long intergenic noncoding RNA (LINC00624) and the coding gene CHD1L, a helicase which binds to PARP1, a known HIV-1 host dependency factor. Infection assays in iPSC-derived macrophages demonstrated increased HIV-1 replication in CHD1L knockdown and knockout cells in a dose-dependent manner. We provide evidence from population genetic and experimental studies suggesting that CHD1L is a novel host HIV-1 restriction factor in human populations. Studies are ongoing to determine the mechanism underlying this important observation.

B078

TMPRSS2 promotes SARS-CoV-2 evasion from NCOA7-mediated restriction

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Abstract

Interferons play a critical role in regulating host immune responses to SARS-CoV-2, but the interferon (IFN)-stimulated gene (ISG) effectors that inhibit SARS-CoV-2 are not well characterized. The IFN-inducible short isoform of human nuclear receptor coactivator 7 (NCOA7) inhibits endocytic virus entry, interacts with the vacuolar ATPase, and promotes endo-lysosomal vesicle acidification and lysosomal protease activity. Here, we used ectopic expression and gene knockout to demonstrate that NCOA7 inhibits infection by SARS-CoV-2 as well as by lentivirus particles pseudotyped with SARS-CoV-2 Spike in lung epithelial cells. Infection with the highly pathogenic, SARS-CoV-1 and MERS-CoV, or seasonal, HCoV-229E and HCoV-NL63, coronavirus Spike-pseudotyped viruses was also inhibited by NCOA7. Importantly, either overexpression of TMPRSS2, which promotes plasma membrane fusion versus endosomal fusion of SARS-CoV-2, or removal of Spike's polybasic furin cleavage site rendered SARS-CoV-2 less sensitive to NCOA7 restriction. Collectively, our data indicate that furin cleavage sensitizes SARS-CoV-2 Spike to the antiviral consequences of endosomal acidification by NCOA7, and suggest that the acquisition of furin cleavage may have favoured the co-option of cell surface TMPRSS proteases as a strategy to evade the suppressive effects of IFN-induced endo-lysosomal dysregulation on virus infection.

B080

Generating stable insect cell lines to study extracellular vesicles in baculovirus infection

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Abstract

Small extracellular vesicles (sEVs) of mammalian cells infected with HIV or herpes simplex virus, among others, were found to illicit pro- or antiviral reactions in non-infected cells. It would be interesting to study if sEVs play a similar role in insect-virus interactions, which can be studied in baculovirus infection of *Spodoptera frugiperda* cells. Firstly, to identify the effect of sEVs from infected cells upon naïve cells, it is essential to separate virions from sEVs. To this end, size exclusion chromatography was combined with sucrose density gradient ultracentrifugation. With no known protein marker for EVs of these insect cells, determination of sEV rich fractions relied on nanoparticle tracking analysis. To overcome this constraint, two stable cell lines, expressing baculovirus membrane glycoprotein GP64 or mammalian tetraspanin CD81 – a protein marker for sEVs – fused to GFP, were created: SfC1B5-GP64 and SfC1B5-CD81-GFP.

Incorporation of the gene expressing GP64 or CD81-GFP into the genome of SfC1B5 cells was confirmed by PCR, and production of the protein by western blotting. Localisation of GP64 to the cell membrane was confirmed by confocal microscopy. Incorporation of GP64 into sEVs is being validated by immunogold electron microscopy. Following sucrose density gradient ultracentrifugation with sEVs of SfC1B5-GP64, a faint signal of GP64 was detected in the top fraction, which correlates to sEVs localisation. Incorporation of CD81-GFP into sEVs will also be validated. Both cell lines should help identify which fraction contains the majority of sEVs, aiding in the separation of sEVs from baculovirus virions.

B081

Characterisation of a novel mechanism of adaptive immune evasion by human cytomegalovirus

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Abstract

Human cytomegalovirus (HCMV) is a ubiquitous and clinically significant herpesvirus, causing substantial morbidity and mortality in immunocompromised individuals and congenital infection in 1/100 pregnancies. HCMV has evolved a myriad of strategies to evade the host immune response, including targeting host proteins for degradation. Insights into this viral antagonism can facilitate the development of novel antiviral therapies by restoring the activity of endogenous proteins important for antiviral restriction and immunity.

A multiplexed proteomic approach identified 35 host proteins that are degraded with high confidence during HCMV infection (Nightingale et al, Cell Host&Microbe 2018). We have already shown that a subset of these proteins are functionally important in innate and adaptive immunity, including known and novel antiviral restriction factors. However, the role of the remaining proteins during HCMV infection is largely unknown. Furthermore, we have previously employed diverse proteomic approaches to predict HCMV genes that target each protein, including interactome analysis and a screen of HCMV mutants with deletions in contiguous gene blocks.

We have now employed an extensive literature search to identify degraded proteins that have novel function in stimulating NK- and T-cell based immunity. My presentation will focus on immunological assays and proteomic approaches used to characterise the mechanisms of function and viral antagonism of a novel host target degraded in the proteasome by HCMV. We will therefore show how characterisation of key cellular proteins degraded during infection provides insights into HCMV biology and the potential for novel antiviral therapeutics.

B082

Impact of dengue virus NS4A mutation on NF-κB pathway inhibition and viral replication in Aedes aegypti mosquito cells

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Abstract

Dengue viruses (DENV) are flaviviruses transmitted by mosquitoes and responsible for the most common and significant acute arthropod-borne viral (arboviral) disease in humans. With up to 100 million infections per year, DENV is the most rapidly increasing arboviral disease and a major public health concern. No antiviral treatments are currently available for DENV, and the licensed vaccine cannot be used in all settings. As such, the development of new vector control strategies is crucial. The immune system of DENV's main vector, *Aedes aegypti*, is a known barrier to transmission that could be manipulated to develop transmission-incompetent mosquitoes. To do so, it is imperative to better understand virus-immune interaction in the vector.

Our previous work demonstrated that DENV-2 and its viral protein NS4A can inhibit the exogenous induction of the NF-κB-regulated immune-deficiency (IMD) signalling pathway. However, we identified specific mutations in the N-terminus of NS4A that abrogate the ability of viral protein to antagonise the IMD pathway. We are testing the relevance of these mutations to a viral context by engineering them into DENV-2 replicons and testing the impact on replication and immune evasion in human and mosquito cell lines.

This knowledge will give us a deeper understanding of DENV's ability to escape the mosquito immune response, which could potentially allow us to develop transmission-incompetent vectors to reduce the burden of dengue disease.

B083

Structural characterisation of Herpes Simplex Virus-1 pUL55

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Abstract

Herpes simplex virus-1 (HSV-1) infects approximately 67% of the human population, causing a lifelong infection and disease that ranges from asymptomatic to severe. This work focusses on pUL55, which is a non-essential tegument protein with poorly characterised functions. Homologues of pUL55 are encoded by multiple alphaherpesviruses including HSV-1, HSV-2 and varicella-zoster virus. As pUL55 lacks identifiable sequence homology with any well-characterised protein family, we sought to characterise its three-dimensional structure in order to gain insights into its elusive function. Bacterially expressed hexahistidine-tagged HSV-1 pUL55 was purified using Ni affinity resin and size exclusion chromatography (SEC). Analysis by multi-angle light scattering (SEC-MALS) suggested that purified pUL55 is in monomer:dimer equilibrium. Purified pUL55 was crystallised and crystals diffracted to 2.5 Å but were highly anisotropic. The lack of identifiable structural homologues precluded structure solution by molecular replacement and experimental phasing attempts were unsuccessful. However, a model of the pUL55 structure generated using AlphaFold2 could be used to successfully solve the crystallographic phase problem, allowing refinement of the pUL55 structure. pUL55 does not share structural homology with any previously determined structures in the PDB, nor any proteins in the entire human proteome as modelled using AlphaFold2, and the crystal structure reveals that pUL55 binds two structural zinc atoms. Identification of pUL55 as a novel zinc-finger protein suggests that it may function as an adaptor protein to bind viral/cellular proteins or nucleic acids. The structure of HSV-1 pUL55 provides a platform for targeted site-directed mutagenesis to probe its function during infection.

B084

Does allelic variation in the IFITM amphipathic helix influence its antiviral activity?

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Abstract

Interferon-induced transmembrane proteins (IFITMs) are host restriction factors that restrict many pathogenic viruses such as influenza A virus, HIV-1, Dengue virus and some coronaviruses. The IFITM amphipathic helix modulates membrane fluidity and curvature and is critical for its capacity to inhibit virus-cell membrane fusion. Previous studies have shown that natural mutations in primate and bat IFITMs modify their antiviral activity by altering localisation and post-translational modifications. Yet how variation in the amphipathic helix influences IFITM function has not been studied. Here we show that the amphipathicity of the IFITM amphipathic helix varies across species and may have functional consequences. By analysing IFITMs from 31 mammalian species, including 17 bat species, we found that bat IFITM amphipathic helices are less amphipathic than those in human. Furthermore, horse IFITM amphipathic helices are more amphipathic relative to that in other mammals. To test whether such variation influences the antiviral activity of IFITMs, we expressed IFITM variants in IFITM1-3 knockout cells and studied viral entry using lentiviral particles pseudotyped with envelope glycoproteins from influenza A virus or SARS-CoV-2. Furthermore, we showed that IFITMs modulate type I interferon production upon poly(I:C) transfection, representing a potentially novel immunoregulatory function. Our results demonstrate that IFITMs can regulate innate immunity in addition to restricting viral entry and reveal inter-species differences among IFITM family members, indicating that functional heterogeneity exists with mammals. These findings may help us understand whether allelic variation in antiviral genes determines the regularity and directionality of zoonotic virus transmissions.

B085

A novel immunotherapy combining *uv*-inactivated reovirus and the targeted agent sorafenib for the treatment of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the most common form of liver cancer and has a 5-year survival rate <20%. Treatment options for HCC are limited to targeted therapies like sorafenib, which have significant toxicity and provide limited survival benefit. Thus, new treatments are needed to improve survival rates for HCC patients and immunotherapies are of growing interest.

Reovirus has been extensively tested as an oncolytic agent but also has potent immune-stimulating properties that highlight it as a potential immunotherapy. Reovirus-induced immune activation occurs independent of replication and *uv*-inactivated reovirus elicits more pronounced interferon responses in some cell types than live virus. Therefore, we hypothesised that *uv*-reovirus induced anti-tumour immunity could improve the response to sorafenib in HCC.

We have applied cellular and molecular methods to analyse models of HCC, both *in vivo* and *in vitro*, to investigate the ability of *uv*-reovirus (or live-reo) to induce anti-HCC immunity when given with or without sorafenib. We found that the combination 'uv-reo+sorafenib' significantly extended the survival of mice with HCC in a T and NK cell-dependent manner. *In vitro*, the interferon response in HCC cells was most strongly induced by *uv*-reo alone or in combination with sorafenib, but not live-reo. This response correlated with enhanced activation of intra-cellular pathways indicative of RLR signalling, but the relative contribution of RIG-I and MDA-5 was variable and treatment-dependent. These data suggest that the *uv*-reo/sorafenib combination, *via* a distinct mechanism, induces productive anti-HCC immunity and might, therefore, have clinical efficacy as an immunotherapy in HCC patients.

B086

Building a foundation for arbovirus-vector interaction studies by generating stable transgenic Aedes aegypti-derived Aag2-AF5 mosquito cell lines.

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Abstract

Aedes aegypti is a major transmission vector for several arthropod-borne viruses (arboviruses), and with global warming and rising insecticide resistance, the increasing prevalence of this mosquito poses a threat to global health. Therefore, it is essential to develop new approaches to limit arbovirus transmission. One such possibility is modifying vector immunity to produce transgenic mosquitoes that limit transmission by suppressing arboviral replication. Antiviral immune responses of mosquitoes include the RNA interference (RNAi) pathway, and NF-κB-regulated Toll and immunodeficiency (Imd) pathways.

Our work aims to develop stable cell lines that facilitate studies into arbovirus-vector interactions based on the A. aegypti-derived Aag2-AF5 cell line. These stable cell lines will help us investigate the function of the flavivirus non-structural protein 4A (NS4A) as an immune antagonist. We previously observed that the dengue virus serotype 2 (DENV-2) NS4A protein inhibits the activation of the Imd pathway from exogenous stimulation. However, these findings were limited because the transient transfection efficiency of Aag2-AF5 cells was only approximately 40%. We show that by generating stable cell lines using zeocin selection, the proportion of protein-expressing cells can be improved to over 95%. Using fluorescence microscopy, we monitored the stability of a control stable cell line expressing GFP, over several passages, once zeocin selection was removed. Overall, our work on flavivirus NS4A and the ability to generate stable cell lines will help improve our understanding of the underlying molecular virus-vector interactions that influence arbovirus transmission. Ultimately, our work takes the first steps towards generating mosquitoes that reduce arbovirus transmission.

B087

PKR-mediated Stress Response Enhances Dengue and Zika Virus Replication

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Abstract

Flaviviruses possess a capped +ssRNA genome but can use cap-independent translation to support their replication. For better understanding this elusive alternative translation we investigate if the cellular environment can promote the switch in viral translation mode. Hence, we explored how the integrated stress response (ISR), which promotes translational arrest by eIF2α phosphorylation (p–eIF2α), regulates Flavivirus replication. During Dengue virus (DENV) and Zika virus (ZIKV) infection, eIF2α activation peaked at 24 hours post-infection and was dependent on PKR but not type I interferon. The ISR is activated downstream of p-eIF2α during infection with either virus, but translation arrest only occurred following DENV infection. Despite this difference, both DENV and ZIKV replication was impaired in cells lacking PKR and this phenotype is independent of IFN-I/NF-kB signalling or cell viability. By using a ZIKV 5'UTR reporter system as a model, we found that this region of the genome is sufficient to promote an enhancement of viral mRNA translation in the presence of an active ISR. Together we provide evidence that Flaviviruses escape ISR translational arrest and co-opt this response to increase viral replication.

B088

Knocking out the IFITM locus in chicken embryonic fibroblast cell line DF-1 increases the yield of influenza and Newcastle disease viruses; a proof of concept for cell-based vaccine production.

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Abstract

The production of many human and livestock vaccines relies on embryonated chicken eggs. However, the global supply of eggs is underpinned by a complex supply chain that makes scale up during a disease outbreak challenging, requires prioritisation between vaccines and geographic areas, and produces vast amounts of biohazardous animal waste. Avian origin immortalized cell lines have the potential to provide an alternative to eggs, but widespread use is currently limited by lower vaccine yields and lack of suitability for specific viruses. Interferon-induced transmembrane protein (IFITM) proteins are now well known as a broad range of viral restriction factors in vertebrates. Therefore, we used CRISPR/Cas9 gene editing to delete the IFITM locus in chicken DF1 cells and examined the impact on viral yield, infectivity, and the abundance of cells expressing the surface immunogenic protein post influenza (AIV) and Newcastle disease virus (NDV) infection. Compared to wild type DF1, AIV yields increased up to 1.5 $\log_{10}$ pfu, and NDV LaSota increased by 0.8 $\log_{10}$ pfu. Both correlated with higher infectivity and higher expression of haemagglutinin and fusion protein. To confirm increased viral yields were not due to unknown effects, we rescued the restriction phenotype in edited cells through transient expression of IFITM genes. Expression of IFITM3 but not IFITM1 restored AIV restriction, while expression of both IFITM1 and IFITM3 restored NDV infectivity. In conclusion, we have confirmed that removing IFITM genes enhances viral growth in DF1 cells, presenting a proof-of-concept technology to increase the value of avian cell-based vaccine production.

B089

Microwave treatment of 3D cell culture models of HPV16 positive keratinocytes can reverse tumorigenesis and inhibit HPV replication

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Abstract

Human papillomavirus (HPV) is the most common sexually transmitted infection globally. Infection in the ano-genital tract with a high-risk virus can result in lesions which can progress, although rarely, into cervical and other anogenital cancers. Current treatments have an associated high pain, recurrence rate and low efficiency. Emblation Limited's Swift Microwave Tissue Ablation system appears a promising solution to these problems and has been successfully used for the treatment of verrucas, caused by low-risk HPV subtypes (>75% success rate compared to > 33% for cryotherapy). Microwave treatment of 3D in vitro grown keratinocyte tissues (HPV16-positive NIKS16 and SiHa cells which resemble an infection and cancer disease model respectively) resulted in cell death in the treated zone while tissue integrity was disrupted in the adjacent area. Proliferation was inhibited (Ki67, MCM2) whilst apoptosis (cleaved caspase 3) and autophagy (LC3) were induced over time across the entire tissue. Thermal (HSP70) and translational stress (G3BP, PABPC1) was also observed. Expression of the HPV16 oncoproteins, E6 and E7, was reduced in cells in the treated and untreated zones alongside a reduction in expression of the late proteins E4 and L1 in NIKS16 tissues. Analysis of key HPV16 transcripts following treatment suggests that the observed reduction in protein is occurring post-transcriptionally. These results suggest that microwave treatment results in a reversal of tumorigenesis and inhibition of HPV replication and when compared to the currently available liquid nitrogen treatment, ablation caused by microwave treatment was found to be more localised and reproducible.

B090

Comparison of two pseudotyping systems for Rift Valley fever virus as a surrogate to live virus in neutralisation assays

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Abstract

Rift Valley fever virus (RVFV) is an arbovirus that can cause severe disease in infected livestock and humans. It is endemic in sub-Saharan Africa, but climate change and other ecological and social factors are increasing the risk of outbreaks outside the current geographical area. This combined with a lack of licensed vaccines and effective therapeutics has led the WHO Research and Development Blueprint to list RVFV as high priority pathogen for research and development towards vaccines, therapies and diagnostic tools. One significant limitation to this is the need for Biological Safety Level (BSL) 3 laboratories for live virus work to be undertaken.

To overcome this limitation, we have produced RVFV pseudotyped virus (PV). The codon optimised RVFV GnGc gene was expressed in two pseudotyping systems, a human immunodeficiency virus (HIV) and a vesicular stomatitis virus (VSV). We compared titres of the two RVFV PVs and their performance in neutralisation assays against a panel of human serum samples to determine which system produced reliable, reproducible results and correlated with live virus-based neutralisation assays. The VSV-based pseudotype system produced RVFV PV stocks with 10-fold higher titre than the HIV-based system and neutralisation assays could be run in half the time (2 days *versus* 4 days). Preliminary neutralisation data suggested that there was a reasonable correlation in antibody titres between PV systems expressing RVFV GnGc. The next steps will be to extend this to other members of the bunyavirales family to support preparedness for outbreaks of known or emerging viruses.

B091

High-throughput screening of an FDA-approved drug library to identify compounds inhibiting orthopneumovirus fusion

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Abstract

Orthopneumoviruses are enveloped negative-strand RNA viruses and include human respiratory syncytial virus (hRSV), bovine (bRSV), and pneumonia virus of mice (PVM). In humans, RSV causes mild, cold-like symptoms; however, it can also induce life-threatening disease, especially among infants and older adults, leading to over 150000 fatalities each year, globally. Currently, no vaccine is available for hRSV, and the sole therapy relies on monoclonal antibodies that block entry. Therefore, identification of efficient compounds to tackle hRSV infection is crucial. The first step of viral infection relies on the interaction of the virus with its specific cellular receptor(s). Upon binding, fusion of the viral envelope with host cell membrane is carried out by the fusion protein (F), the essential structural protein for entry. From an infected cell, pneumoviruses can spread to neighbouring cells by inducing fusion of plasma membranes due to the expression of the viral glycoproteins at their surface, leading to formation of syncytia.

In our study, we developed high-throughput cell-cell fusion assays for hRSV and bRSV, based on the stable expression of split eGFP/luciferase reporters and doxycycline inducible expression of viral F proteins. These systems were used to screen for inhibitors of viral fusion using an FDA library containing more than 2700 different compounds. Biologically relevant hits involved in pathways such as autophagy and ion channel activity were further validated using recombinant RSV expressing GFP, identifying compounds that can act as broad range inhibitors of pneumovirus infection.

B092

Towards a universal rhinovirus vaccine: Characterisation of antibody responses induced by conserved capsid epitopes

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Abstract

Rhinovirus (RV) is responsible for 70% of respiratory tract infections and is estimated to cause losses of $40 billion annually to the US economy alone. Despite this, it has not yet been possible to develop an effective rhinovirus vaccine, due to the many different serotypes (approximately 150) which generate little or no cross protection. However, antibodies generated directly against the highly conserved capsid protein VP4, can neutralise different serotypes. We have previously shown only antibodies that target the first 45 N-terminal amino acids are neutralising. This makes the N-terminus of VP4 an ideal candidate for a universal rhinovirus vaccine. Here we have investigated the ability of experimental RV VP4 N-terminal peptide vaccines to initiate an immune response in mice. We have compared three different sized peptides (1-15, 1-30 and 1-45 amino acids) and two different peptide conjugation VLP systems (keyhole limpet hemocyanin (KLH) or SpyCatcher). Mice received three doses of either a control VLP or a peptide conjugated VLP and were bled two weeks after each dose. Sera was then analysed by ELISA to assess VP4 antibody responses. This revealed that that the 30 amino acid VP4 peptide conjugated to SpyCatcher produced the most reliable and quickest antibody responses. This will be a good candidate for further RV vaccines studies in a mouse challenge model.

B093

Generating a Universal Platform for Vaccine development using Antigen-Capturing VLPs

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Abstract

Whilst existing vaccine strategies have made enormous impacts on human and animal health, virus-like particles (VLPs) could offer an improved and safer platforms for the rapid development of new vaccines. VLPs are highly immunogenic, yet lack the genetic information required to cause an infection. Additionally, VLPs can be modified as generic vaccine presentation platforms to present a variety of different antigens.

We are studying a novel and flexible VLP presentation platform based on a fused dimer of the hepatitis B core (HBc) antigen, which incorporates an antigen-capturing system. This system is an Affimer which has been isolated to bind a SUMO tag fused to a target antigen, thus creating particles which can capture multiple copies of the target antigen at their surfaces. The multimeric display of antigen proteins at the surface of recombinant VLPs can significantly improve their immunogenicity.

Here, we have produced HBc VLPs, modified to display an anti-SUMO tag Affimer, in the yeast *Pichia pastoris* and determined their capacity to bind a number of antigens bearing the SUMO tag, including the SARS-CoV-2 spike protein. High-resolution cryo-electron microscopy analysis showed that these modified particles assemble into both T=3 and T=4 conformations, similar to wild type HBc particles and reveal clear Affimer density. Through the generation of these VLPs, we have established a pipeline to pave the way for the production of highly immunogenic and stable VLPs, capable of displaying SUMO-tagged antigens required for vaccination.

B094

Advances in HBcAgs VLPs vaccine platforms: generation of a novel capturing system

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Abstract

Generating a fast and flexible system to produce highly immunogenic vaccines able to induce long-lasting protection against emerging and re-emerging pathogens is highly desirable. The hepatitis B core antigen virus-like particle (HBc VLP) platform could be exploited to generate such a system. We have previously modified these VLPs to present adaptors (e.g. nanobody or Affimer) to capture and display tagged foreign antigens. Here, we are adapting the system to display trimeric viral glycoproteins by utilising the C-terminal sequence from T4 bacteriophage fibritin (foldon) to both enhance trimerisation of the target glycoprotein antigen and function as a capture tag to facilitate its binding to modified HBc VLPs. Many viral glycoproteins, such as the SARS-CoV-2 spike protein, function as trimeric complexes and introduction of the foldon sequence can enable such proteins to readily adopt a native conformation. Foldon sequences produced by bacterial expression or by peptide synthesis were used as targets against a library of Affimers via phage display technology. This approach selects high affinity Affimers that recognise foldon in its trimeric form. Selected Affimer sequences will then be fused to the N-terminus of the VLPs. Purified anti-foldon VLPs are characterised using western blot, dynamic light scattering (DLS), and negative stain electron microscopy. These VLPs can then be used to display a range of different antigens in order to generate a flexible vaccine display platform.

B095

The use of microRNA inhibition to produce influenza A virus high growth reassortants for use as candidate vaccine viruses

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Abstract

Influenza viruses cause a significant disease burden, including thousands of deaths globally each year. Due to the virus's ability to rapidly mutate and evade existing immunity, candidate vaccine viruses (CVV) must be updated each flu season to match the current circulating influenza strains and grow sufficiently well to generate enough vaccine. Virus yield can be improved by co-infecting wild-type (WT) viruses with a high-yielding vaccine strain, A/Puerto Rico/8/34 (PR8), to allow the reassortment of virus gene segments and the generation of a high growth reassortant (hgr) virus, that has the haemagglutinin (HA) and neuraminidase (NA) from the WT circulating strain. Traditionally, neutralising antibodies are used to remove viruses possessing the HA and NA of the PR8 donor, however this process can be inefficient and time-consuming.

This project seeks to generate an attenuated PR8 virus by insertion of microRNA target sequences, which are highly expressed in embryonated hens' eggs, into the HA and NA genes by reverse genetics. The miR-HA-NA PR8 virus will be generated in cells lacking the chosen microRNA. Following mixed infection in eggs with WT seasonal viruses, the internal gene segments of the miR-HA-NA PR8 virus will be unhindered and free to reassort, whilst the HA and NA genes will be destroyed by microRNA targeting. This species-specific attenuation may negate the use of custom antibodies, and thereby reduces the need for animals required for their generation. Application of this technology will be compared to the classical reassortment method, assessing feasibility and timeliness of CVV generation.

B096

Trivalent MVA-based vaccine candidate against Ebola, Lassa fever and Marburg virus elicits robust humoral immune response against all three viruses

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Abstract

Ebola, Lassa fever and Marburg virus cause severe diseases often with fatal outcomes. Besides millions of people in Africa living at risk of acquiring an infection with one of these viruses, preventative measures are limited. In terms of production costs and delivery to people with limited access to health care a trivalent vaccine is preferable. However, multivalent designs are associated with the concern of reduced immunogenicity of the individual antigens.

We designed a trivalent MVA-based vaccine candidate to elicit protection against all three viruses and evaluated its immunogenicity in female Balb/c mice. We compared the effect of administrating each antigen alone, the three antigens as a mix of monovalent constructs, and all three antigens encoded together on the trivalent constructs.

After three immunisations, there were no significant differences in the antigen-specific binding antibody titres of animals that received the trivalent construct versus animals which received the respective antigens alone. These results were reflected in the pseudovirus neutralisation assays, where overall neutralising responses were modest. In terms of T cell responses, a significant difference between the magnitude of the response towards the Lassa antigen versus the Marburg and Ebola antigen was observed.

We showed that a multivalent vaccine candidate induces a robust antibody response against several antigens. Cellular immunity appears to be more affected by a multivalent approach. Given the immune correlates of protection of the diseases, future studies aim to investigate if this single vaccine is able to protect from different Filoviruses, as well as Lassa fever virus challenge.

B097

Investigating the antigenic diversity of H5 clades of avian influenza viruses using antigenic cartography and putative antigenic epitopes conferring vaccine efficacy in chickens

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Abstract

Avian influenza viruses (AIVs) continue to burden the poultry industry globally with the H5 subtype dominating outbreaks. Introductions of H5 AIVs into poultry often emerge as highly pathogenic avian influenza (HPAI), devastating poultry populations and posing huge socio-economic consequences. Thus, control measures are driven by vaccination, to reduce morbidity and mortality within the infected populations.

However, the extensive diversity of H5 confounds protective efficacies of previously protective vaccines. 2014 saw the emergence of an antigenically distinct clade, 2.3.4.4, characterised by unique antigenic epitopes and the recombination with various neuraminidase. Consequently, current vaccines for poultry are vastly outdated and not antigenically matched to circulating strains, which may additionally play a role in the evolution and emergence of antigenic variants of H5 AIVs.

This study sought to investigate the antigenic diversity across the antigenic clades of H5 and the key antigenic epitopes responsible for the vaccine failure in chickens following infection of HPAI H5 AIV.

Candidate viruses from recently circulating clades of H5 AIVs were raised as inactivated adjuvanted vaccines in white leg-horn chickens and sera extracted 42 days post-vaccination. Antisera was tested by haemagglutination inhibition assay (HI), and titres plotted using antigenic cartography. Clade 2.3.4.4 was antigenically distinct, with little cross-reactivity between heterologous antisera to the viruses. Therefore, current work investigates putative antigenic epitopes predicted by comparing genetic diversity and antigenic distance established by the antigenic cartography. These results highlight the urgent demand for new vaccines to control AIV globally in poultry populations and reduce the risk of zoonotic emergence.

B098

Differential susceptibility of SARS-CoV-2 in animals: Evidence of ACE2 host receptor distribution in companion animals, livestock and wildlife by immunohistochemical characterisation

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Abstract

Angiotensin converting enzyme 2 (ACE2) is a host cell membrane protein (receptor) that mediates the binding of coronavirus, most notably SARS coronaviruses in the respiratory and gastrointestinal tracts. Although SARS-CoV-2 infection is mainly confined to humans, there have been numerous incidents of spillback (reverse zoonoses) to domestic and captive animals. An absence of information on the spatial distribution of ACE2 in animal tissues limits our understanding of host species susceptibility. Here, we describe the distribution of ACE2 using immunohistochemistry (IHC) on histological sections derived from carnivores, ungulates, primates and chiroptera. Comparison of mink (*Neovison vison*) and ferret (*Mustela putorius furo*) respiratory tracts showed substantial differences, demonstrating that ACE2 is present in the lower respiratory tract of mink but not ferrets. The presence of ACE2 in the respiratory tract in some species was much more restricted as indicated by limited immunolabelling in the nasal turbinate, trachea and lungs of cats (*Felis catus*) and only the nasal turbinate in the golden Syrian hamster (*Mesocricetus auratus*). In the lungs of other species, ACE2 could be detected on the bronchiolar epithelium of the sheep (*Ovis aries*), cattle (*Bos taurus*), European badger (*Meles meles*), cheetah (*Acinonyx jubatus*), tiger and lion (*Panthera spp.*). These results provide anatomical evidence of ACE2 expression in a number of species which will enable further understanding of host susceptibility and tissue tropism of ACE2 receptor-mediated viral infection.

B099

Development of a real-time loop-mediated isothermal amplification (LAMP) method for detection of Japanese Encephalitis virus (JEV)

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Abstract

Japanese encephalitis virus (JEV) is a zoonotic mosquito-borne flavivirus, occurring in nature in an enzootic cycle between mosquitoes and birds or livestock (primarily pigs) that act as amplifying hosts. However, there is spillover into other mammals and humans, and JEV is the principal cause of human viral encephalitis in Asia. Rapid diagnosis of JEV infections in livestock or mosquitoes is therefore essential to limit the risk of spillover to humans. Loop-mediated isothermal amplification (LAMP) offers a rapid and simple means of detecting pathogens in biological samples, without the need for thermal cycling associated with polymerase chain reaction methodology.

This study aimed to develop a real-time LAMP assay for diagnosis of JEV infection in pigs and mosquitoes. A combination of four or eight JEV-specific primers were assessed for sensitivity and specificity using real-time LAMP. Reactions were maintained at 65°C for one hour followed by melting curve analysis to identify the dissociation temperature.

The assay detected JEV RNA derived from the envelope gene within 45 minutes, with results confirmed by melt curve analysis. The specificity was 100% with clear differentiation between JEV and related flaviviruses including WNV and USUV. Sensitivity was lower than for RT-PCR with a limit of detection of 84.9 ng/µl RNA, although further analysis is required to confirm these findings. However, the assay was effective in detecting JEV RNA within infected mosquito bodies and salivary glands, and could be adapted for rapid diagnosis of JEV infection in locations outside of the laboratory as a field-based screening assay.

B100

Decay of infectious SARS-CoV-2 and MHV in wastewater

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Abstract

Sewage spill-over into water bodies raises significant public health concerns, especially in light of recent studies revealing the presence of virus in the stool of individuals infected with severe acute respiratory coronavirus 2 (SARS-CoV-2). Our recent studies have demonstrated that SARS-CoV-2 infectivity decays rapidly in freshwater and seawater, whereas viral RNA is stable for long periods of time in these environments (Sala-Comorera *et al*., 2021). However, currently our knowledge of SARS-CoV-2 persistence in faecally contaminated water and wastewater, especially virus viability, is lacking. Furthermore, the absence of a suitable low containment model system has hindered our current understanding of SARS-CoV-2 in the environment, therefore we compared decay rates of SARS-CoV-2 with another beta-coronavirus, Mouse Hepatitis Virus (MHV), as a potential surrogate virus that can be handled in BSL-2 containment. We will present the findings of a recent study where we determined the decay rates of viable infectious SARS-CoV-2 and MHV, and viral RNA, in a wastewater microcosm at various temperatures. Our findings indicated that viral RNA amplicons were stable for the duration of the experiment and suggest minor degradation over multiple days. Cytopathic effect was evident in samples incubated with SARS-CoV-2 and MHV at all temperatures after 48 hours. Furthermore, viable SARS-CoV-2 and MHV were also detectable after 140 hours at 4 °C. The decay rates in wastewater of intact and free RNA derived from SARS-CoV-2 and MHV will also be described. Together these results will further inform wastewater monitoring in the current pandemic response and in future outbreak mitigation.

B101

Molecular characterisation and diagnostic development for Oropouche fever, a neglected tropical viral disease

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Abstract

Oropouche fever is a neglected tropical viral disease endemic to Latin America and the Caribbean with potential to cause future pandemics. Oropouche fever is caused by Oropouche virus [SCG1] (OROV), an orthobunyavirus with a tri-segmented (-)ssRNA genome and a propensity for genetic reassortment. OROV commonly infects sloths, birds, monkeys and rodents but has been known to spill over into human populations *via* transmission from biting insects.

After Dengue fever, Oropouche fever is the second-most prevalent insect-borne disease in Brazil. Over 500,000 infections have been documented as the result of numerous regional epidemics in South America since its discovery in 1955. Changes in climate and land use are increasingly bringing humans into contact with OROV reservoir and vector species, but currently there is no readily available point-of-care diagnostic test for OROV infection.

By producing high-quality OROV antigens and anti-OROV nanobodies we aim to (1) interrogate the immune response to OROV infection, (2) develop cost-effective, in-field diagnostics, and (3) probe the use of recombinant OROV surface antigens as vaccine candidates. Preliminary progress on OROV antigen generation and characterisation will be discussed.

B102

Evaluation of the efficacy of a quaternary ammonium compound antimicrobial coating on public transport surfaces using Phi6 as a surrogate for SARS-CoV-2

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Abstract

Antimicrobial coatings could be a good way of reducing fomite transmission. If they are proven to be effective at reducing viral concentration on surfaces they could contribute to minimising SARS-CoV-2 transmission on public transport.

Metal and plastic tray tables from trains were coated with a quaternary ammonium compound antimicrobial coating in the laboratory. Fetal bovine serum (FBS) was added to simulate dirty conditions and form a more realistic scenario. Over 4 weeks survival of Phi6, a surrogate for SARS-CoV-2, on coated and uncoated tables with and without FBS, was assessed over a 2h contact time. Plaque assays were performed to determine pfu/ml. Efficacy was calculated by subtracting the LOG10 of the average pfu/ml on the coated tables from the LOG10 on the uncoated tables (n=3).

On uncoated tables, regardless of the presence of FBS, there was little reduction in Phi6 concentration over 2 hours but Phi6 concentration was reduced on coated tables. The coating appeared to be more effective on the metal table with an efficacy of 6.7-7.6 LOG10 reduction each week, compared to the efficacy of 4.3-7.5 LOG10 reduction on the plastic table. However, with the introduction of FBS, efficacy fell to between 0.1-1.6 LOG10 reduction on the metal table and 0.1-1.1 LOG10 reduction on the plastic table.

This work demonstrates how soilage affects the efficacy of antimicrobial coatings. It highlights the importance of continued cleaning for the coating to reduce virus survival on surfaces but the effect of cleaning on the coating remains to be investigated.

B103

Detection of SARS-CoV-2 in Northern Ireland Wastewater

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Abstract

SARS-CoV-2 is shed in high levels in the faeces and urine leading to wastewater-based epidemiology (WBE) becoming an integral part of the response to the global pandemic. SARS-CoV-2 is shed by asymptomatic, symptomatic and pre-symptomatic individuals, meaning that wastewater testing can be used as an early warning sign for outbreaks in certain geographical areas. The potential to assess infection levels in large populations as well as being considerably cheaper and less invasive than clinical testing further contributes to WBE's importance during the pandemic. Multiple countries, including those found in the rest of the United Kingdom, have active WBE programmes. Throughout Northern Ireland (NI), thirty-three Wastewater Treatment Inlets are part of a routine screening programme for SARS-CoV-2 in wastewater. These sites capture 62% of the NI population, which gives an important snapshot of infection levels among the NI population in different localities. Data generated has been vitally important to the public health response and stakeholder decision making within NI. Optimising processing methods is important in ensuring that samples are not below our limit of detection and therefore inaccurately declared negative. In this study we evaluate numerous wastewater processing methods to ensure maximum viral recovery from each sample.

B105

Examination of *in vitro* cell lines and *ex vivo* tracheal organ cultures for the study of porcine respiratory coronavirus.

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Abstract

Porcine respiratory coronavirus (PRCV) is a highly prevalent pathogen endemic to many countries, including the UK. It is the causative agent of mild respiratory disease in pigs, however the pathology observed in PRCV infected lung tissue has been reported to compare to that observed in humans infected with severe acute respiratory syndrome related coronavirus (SARS-CoV). SARS-CoV and PRCV infection share common features including prominent lower respiratory tract disease, the disruption of bronchial and alveolar epithelium, lesions, pneumonia and necrosis. These observations have prompted greater interest into the study of PRCV.

We have investigated the *in vivo* phenotype of four strains of PRCV: 86/135308 (135), 86/137004 (137), AR310 (310) and ISU-1. Disparities in both the quantity of infectious viral progeny and PRCV derived RNA were observed in the trachea and lung, with titres of 135 and 137 higher than both ISU-1 and 310. Titres were comparable in nasal swabs, nasal turbinates', and bronchoalveolar lavage fluid. *In vitro* replication in ST (porcine swine testis) and LLC-PK_1 (porcine kidney) continuous cell lines however did not identify any differences in either productive infection or RNA replication between the four strains. Differential replication was observed in *ex vivo* tracheal organ cultures (TOCs), providing comparable data to that observed from tracheal samples infected *in vivo*. These findings have indicated *ex vivo* TOCs may be a representative alternative to *in vivo* infection for the study of PRCV in line with the guiding principles of the Three Rs for the replacement, refinement, and reduction of animal resources.

B106

Tools to investigate the interactome of the influenza A virus polymerase in replication

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Abstract

A set of host cell factors are essential to support the replication and life cycle of influenza A virus, which is a significant pathogen responsible for both seasonal epidemics and occasional pandemics. Many influenza virus-host interactions involve the influenza virus polymerase, which carries out both replication and transcription of the influenza A virus genome. For example, two members of the family of ANP32 proteins, ANP32A and ANP32B, serve essential but redundant roles in supporting influenza polymerase activity in human cells. In human cells that lack expression of ANP32A and ANP32B, incoming influenza genomes are transcribed into mRNAs but not replicated. While host factors that are important for the influenza virus life cycle have been identified in genetic screens, which of the virus-host interactions specifically required for either transcription or replication by the influenza virus polymerase remain unclear. Using a proteomics-based approach, our work aims to identify host factors that play a specific role in influenza A virus replication. The use of influenza A virus with a tagged polymerase will allow affinity purification and identification of proteins interacting with the polymerase in the context of live virus. To specifically understand the interactome of the polymerase in replication, we compare the interactome of the influenza polymerase in wild-type human cells and ANP32-knockout cells, which act as a model for restricted replication. Gaining insight into the specific role of host factors in influenza A virus replication is important to elucidate the mechanisms behind influenza virus pathogenesis and identify new therapeutic targets.

B107

Superinfection exclusion maintains spatially distinct influenza virus populations

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Abstract

Influenza viruses can interact during coinfections. These interactions affect viral fitness through genome complementation and competition, and increase population diversity through reassortment. However, opportunities for interactions are limited as coinfection is blocked a few hours after primary infection by a process known as superinfection exclusion (SIE). We investigated how SIE at the level of individual cells impacts the spatial patterning of influenza virus infections. We created a simplified model where monolayers of cells were infected with two isogenic influenza A viruses which encoded different fluorophores, allowing easy identification of coinfected cells. We showed in this system that SIE onset occurs within 4 hours of primary infection independent of the amount of secondary virus, revealing a limited role for direct competition between coinfecting viruses. We then asked if SIE would create patterns of coinfection as viruses spread through a monolayer of cells under plaque assay conditions. We observed that plaques that grew from a coinfected focus did not segregate into areas where one virus dominates, as all new infections were of cells that had not yet established SIE. In contrast, plaques that grew into each other from separate foci established only minimal regions of overlap before SIE blocked further coinfection. In this way, the kinetics of SIE onset results in discrete regions of infection, within which genome interactions can occur freely, and between which they are blocked. This implies that the interactions which drive influenza population diversity and fitness will be strongly influenced by the spatial patterning of infections within hosts.

B108

Different requirements of CDK9 in host cell and Herpes Simplex Virus transcription.

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Abstract

All herpesvirus gene expression requires active recruitment of the host RNA polymerase II and components of transcriptional apparatus to the virus genome. Notwithstanding considerable insight from previous studies, our understanding of precisely how the virus promotes such recruitment and selective activity of the host transcriptional apparatus remains incomplete. We performed high resolution spatial analysis of Herpes simplex virus transcriptional output in combination with click chemistry for simultaneous genome detection to study the involvement of the host transcriptional machinery at the earliest stages of infection. One of the key control points in host cell transcription is the regulated release of RNA Pol II from pausing catalysed by P-TEFb. We used DRB, a selective inhibitor of P-TEFb to investigate its requirement for HSV transcription. We find that whilst within 3 hours of addition, DRB strongly suppressed viral immediate early (IE) protein synthesis it did not prevent the initiation of IE gene transcription and progression to the distal regions of the genes. Rather we find that in contrast to control conditions, where initial IE bursting had subsided and the majority of IE transcripts were transported to the cytoplasm, DRB induced the presence of large IE nuclear transcript foci colocalised with the HSV genome, with transcripts failing to release from the genome and being heavily restricted to the nucleus. These data suggest that RNA Pol II pause-release by P-TEFb/CDK9 is not critical for IE transcription but is involved later in the transcriptional process, for proper processing and transport of RNA away from the genome.

B109

Positive strand RNA viruses differ in the constraints they place on the folding of their minus strand

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Abstract

Genome replication of positive strand RNA viruses requires the production of a complementary negative strand RNA that serves as a template for synthesis of yet more positive strand progeny. Structural RNA elements are important for genome replication, but while they are readily observed in the positive strand, evidence of their existence in the negative strand is more limited. We hypothesised that this was due to viruses differing in their capacity to allow this latter RNA to adopt structural folds. To investigate this, ribozymes were introduced into the negative strand of different viral constructs. Our expectation was that if RNA folding occurred, negative strand cleavage and suppression of replication would be seen. Indeed this was what happened with hepatitis C virus (HCV) and feline calicivirus constructs. However, ribozymes had a much more limited impact on the replication of hepatitis E virus and rhinovirus constructs, and were without effect when introduced into chikungunya virus and yellow fever virus constructs. Reduced cleavage in the negative strand proved to be due to duplex formation with the positive strand. Interestingly, ribozyme-containing RNAs also remained intact when produced *in vitro* by the HCV polymerase, again due to duplex formation between the nascent and template strands. Overall, our results show that the structural state of the negative strand fundamentally differs between different positive strand RNA viruses.

B110

Characterisation of Influenza A virus (IAV) segments enriched in CpG or UpA dinucleotides through synonymous genome recoding

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Abstract

Synonymous viral genome recoding alters the nucleotide, dinucleotide, codon or di-codon composition of a viral gene while retaining its coding sequence. This technique has been used to generate attenuated viruses that are potential vaccine candidates including poliovirus, respiratory syncytial virus, and influenza A virus (IAV). However, the mechanistic determinants for how the synonymous mutations attenuate viral replication are not well understood. Recoding could sensitise the viral RNA to antiviral proteins such as ZAP or RNase L as well as the no-go decay translation quality control pathway.

IAV has a segmented genome composed of eight negative-sense RNA molecules (vRNAs). We synonymously recoded segments 1 to 6 of two IAV isolates to maximise their CpG and UpA dinucleotide contents. Expression of each recoded segment was tested in a minireplicon assay whereby plasmids coding for the viral RNA-dependent RNA polymerase and nucleoprotein are co-transfected with a plasmid coding for a vRNA. This platform allows for rapid screening of modified segments. We found that maximising the CpG or UpA dinucleotide content of the IAV segments inhibited viral gene expression, from moderate decreases to abolishing it completely. High UpA content had the most deleterious effect. Recoded segments are being assessed for changes in splicing, translation, and sensitivity to antiviral proteins. The effect of CpG and UpA enrichment on viral replication will be assessed for a subset of the recoded segments.

Determining how synonymous genome recoding mechanistically inhibits viral replication will allow rationally designed novel live attenuated virus vaccines to be developed.

B111

The use of novel tools to uncover the ultrastructure of the FMDV replication complex by correlative light-electron microscopy.

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Abstract

Foot-and-mouth disease virus (FMDV) is a veterinary pathogen of major global concern, due to the economic impact of sanctions imposed during disease outbreaks. FMDV, like many positive sense RNA viruses, reorganises cellular membranes to form viral replication complexes (RCs). The detailed structure of FMDV RCs is poorly characterised. Here we are using a replicon system which allows replication to be studied without high containment or the complexity of other parts of the viral lifecycle. To study the RCs, we have developed a number of modified replicons, incorporating tags for use in room temperature correlative light electron microscopy (CLEM). Osmium-resistant fluorescent protein, mEosEM has the advantage of maintaining its fluorescence after osmium tetroxide staining, which greatly increases sample contrast for EM. We have generated a replicon containing mEosEM and shown that the fluorescent signal is detected by live cell and confocal imaging and within sectioned resin-embedded cells. This allows us to identify cells where active viral replication is taking place. In addition, metallothionein tags bind gold nanoparticles to form detectable dense clusters of a regular size providing a specific signal for imaging by electron microscopy. We have generated a replicon with an MT tag fused to one of the three 3B proteins, which locates within the RCs, and demonstrated that this is still replication-competent. Use of metallothionein tags allows us to locate 3B within RCs, unravelling its molecular arrangement during viral replication. These tools are allowing us to elucidate the ultrastructure of FMDV RCs and enhance our understanding of FMDV replication.

B112

Regulation and Expression of Gene transfer agents: Another step towards a mechanistic understanding.

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Abstract

Gene transfer agents (GTAs) are like a defective phage that can package and transfer a random piece of the producing cell's genome, but are unable to transfer all the genes required for their own production.

The recent discovery that the product of gene rcc01865 is a specific GTA activation factor (gafA) and essential for GTA production in *Rhodobacter capsulatus* (RcGTA), has improved our understanding of the mechanisms for production of RcGTAs.

However, the complete mechanism remains unclear; so our goal was to further investigate the mechanisms.

So far we have used the Bacterial Two-Hybrid System to show protein-protein interaction occurs between the RNA polymerase omega subunit rpoZ and gafA. We quantified the interaction between the two proteins by measuring β-galactosidase activity in liquid cultures. Further evidence for the interaction has been gained through site directed mutagenesis of the N- and C-terminal regions of gafA.

Construction of targeted mutants and trans-complementation studies were carried out. These were quantified by GTA transduction assays. The results of which were verified by Western blots using capsid protein anti-serum.

Our results will boost our understanding of this fascinating type of horizontal gene transfer; with all of its impact upon bacterial evolution and antimicrobial resistance. Not only in the model species but help in the detection of GTAs in other species that hold gafA homologues.

B113

Imaging of dynamic Chikungunya virus RNA replication elements by electron microscopy

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Abstract

Chikungunya virus (CHIKV) is a re-emerging, pathogenic *Alphavirus* transmitted to humans by *Aedes spp* mosquitoes. Since ~2000 CHIKV has undergone epidemic spread from previously endemic areas of Africa and South-East Asia into India, China, Southern Europe and the Americas. CHIKV infection is associated with very high rates of morbidity and pain, associated with a range of both acute and chronic arthralgia symptoms. Despite this, no antiviral therapy or vaccine has been licensed.

We previously demonstrated that the 5’ untranslated region (5’UTR) in the RNA genome of CHIKV folds into a number of dynamic RNA structures and interactions that have been proven to be essential for viral genome replication. This was proven using a combination of reverse genetics and SHAPE mapping which is a widely used biochemical technique to define RNA structures at single-nucleotide level, by exploiting small electrophilic reagents that react with the 2’ hydroxyl group present within the nucleotides.

In the current study, we present 3D class average reconstructions of the complex RNA replication elements derived from negative staining electron microscopy (EM). The RNA was obtained from the CHIKV-Fluc replicon using in vitro transcription and was folded in optimal conditions previously developed. We also present optimisations of cryo-electron microscopy to investigate these essential dynamic RNA interactions in molecular detail. With these results we hope to further investigate the replication process of CHIKV and more specifically we aim to understand how the 5’ UTR interacts with cellular and viral proteins.

B114

Investigating the potential role of Cyclophilin A in Hepatitis E virus replication

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Abstract

Hepatitis E virus (HEV) is a single-stranded positive-sense RNA virus and a leading cause of acute hepatitis, responsible for >20 million cases per year. In healthy individuals, infection is typically self-limiting, however in certain groups mortality rates of up to 30% have been recorded. Currently, there are no specific treatments or vaccines to combat HEV infection approved outside of China. Therapeutic targets must therefore be identified to aid in the development of treatments against HEV infection.

Replication of a number of RNA viruses, including related hepatotropic viruses such as hepatitis C virus, has been shown to require the involvement of the peptidyl-prolyl cis/trans isomerase cyclophilin A (CypA). CypA therefore represents a possible pan-viral therapeutic target. However, the involvement of CypA in HEV replication has yet to be unambiguously defined.

To address this question, we first assessed the effect of pharmacological inhibition of CypA with the potent inhibitor cyclosporin A (CsA) on HEV genome replication using a subgenomic replicon system, with replication monitored across several days at varying drug concentrations. We found that non-cytotoxic concentrations of CsA had no effect on the replicative capacity of HEV. To confirm these observations we depleted endogenous CypA using shRNAs. Consistent with the lack of pharmacological inhibition we found that CypA knock-down also had no effect on HEV genome replication. We conclude that unlike other positive-sense RNA viruses, HEV genome replication is independent of CypA, highlighting the need to further study the replication mechanisms of this virus.

B115

Investigation of non-canonical roles of CHIKV nsP1 through the dissection of an auxiliary methionine.

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Abstract

Chikungunya virus (CHIKV) is a single-stranded, positive-sense RNA virus of the *Alphavirus* genus. CHIKV is an arbovirus, whose spread is mediated by *Aedes* species mosquitos and is associated with debilitating joint pain and febrile symptoms in infected humans. A lack of vaccine or specific antivirals, combined with increasing global spread, has facilitated the re-emergence of CHIKV in recent years. Our research focuses on the CHIKV encoded non-structural protein-1 (nsP1), which has methyltransnferase activity and is essential for virus genome replication. Through a combination of structural, biochemical and reverse genetic approaches, we aim to investigate the relationship between the molecular structure and both canonical and non-canonical functions of nsP1, at different stages of CHIKV replication.

We have introduced a panel of substitutions at an in-frame methionine (M^{24}) in nsP1 in order to determine the importance of this residue within the CHIKV replication cycle. Utilising reverse genetics, we have demonstrated that this residue is critical for efficient CHIKV replication and that substitutions inhibit production of infectious virus. Importantly, we have shown that these changes have no significant effect on sub-genomic replicon replication but effect viral titres following infection; suggesting that nsP1 has roles in the later stages of virus replication; such as packaging or egress.

B116

Hepatitis E virus replication is controlled by a novel mechanism of polyprotein processing

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Abstract

Hepatitis E virus (HEV) is a leading cause of acute viral hepatitis worldwide. As well as faecal-oral transmission between humans, the virus can be transmitted zoonotically. Zoonotic transmissions are of particular concern and are associated with increased mortality in some groups. Currently, there are no strategies to treat HEV infection, in part due to a poor understanding of many aspects of virus biology.

HEV has a positive-sense single-stranded RNA genome encoding a polyprotein (termed pORF1), required for genome replication. It is predicted to contain seven domains based on sequence homology to related viruses. It is postulated that the viral polyprotein is processed to separate these domains into individual functional units (sometimes called replicase or non-structural proteins). However, it is unknown if the HEV polyprotein undergoes proteolysis, the mechanism that underpins any proteolysis or what the functional units of replication actually are.

Using a combination of *in vitro* translation, proteolysis and replication assays, our data suggest that, in contrast to related RNA viruses, HEV pORF1 has no auto-catalytic activity, suggesting a cellular factor is important for pORF1 processing. In the presence of thrombin, we show that pORF1 undergoes specific proteolysis to produce distinct protein products. Using mutagenesis we show that this proteolysis occurs at defined sites which match the thrombin recognition consensus and are highly conserved. Mutagenesis prevents viral genome replication, as does pharmacological inhibition of thrombin. We propose that HEV has developed a unique strategy for genome replication that could be important for controlling tropism and zoonosis.

B118

An integrative workflow using cryo-CLEM to investigate the structure of the HCV replication complex

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Abstract

Hepatitis C virus (HCV) affects an estimated 71 million people worldwide and is a major cause of chronic liver disease. HCV infection induces rearrangements of cytoplasmic membranes, including the production of double membrane vesicles (DMVs), proposed as the sites of HCV genome replication. However, knowledge is lacking on how the viral non-structural (NS) proteins assemble within DMVs.

To gain understanding of the arrangement of NS proteins within DMVs, we aimed to setup a cryo-correlative light and electron microscopy (cryo-CLEM) workflow. For this we have used Huh7 cells stably harbouring an HCV subgenomic replicon in which NS5A is fused with EGFP, to investigate the morphology of DMVs and their interactions with NS5A and other HCV NS proteins. However, the EGFP insertion is only tolerated in the C-terminal domain III of NS5A, preventing co-localisation of NS5A with other tagged NS proteins. To address this, we have employed Versatile Interacting Peptide tags miniE and miniR (VIPER), which exploits a coiled coil heterodimerisation of the two peptides. We demonstrate that the genetic miniE tag is also tolerated in NS5A domain III – allowing detection using a fluorescent miniR probe. We are currently assessing whether the miniE tag can be inserted into other NS proteins allowing simultaneous detection of multiple NS proteins in the context of cryo-CLEM.

Findings from our study from the combination of VIPER technology and cryo-CLEM will allow us to unravel the molecular organisation of the HCV replication complex.

B119

Tools to investigate the dynamic composition of viral replication factories

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Abstract

Rotaviruses (RVs) cause life-threatening gastroenteritis in children under 5 years old, particularly in developing countries. To date, there are no efficient anti-RV therapeutics. Replication of the RV genome is believed to occur within cytoplasmic replication factories, termed viroplasms. Recently, we have shown that RV viroplasms represent protein-RNA condensates formed via liquid-liquid phase separation (LLPS) of viral proteins. We have examined a number of small molecules known to disrupt LLPS and identified propylene glycol (PG) as a compound with low toxicity suitable for dissolving viroplasms. We have shown that PG treatments of RV-infected cells significantly reduced the viral titres approximately 150-fold compared to the untreated samples. Dissolution of viroplasms also resulted in dephosphorylation of the viroplasm-forming protein NSP5 that could be reversed by inhibiting cytoplasmic dephosphatases. Interestingly, during late infection stages (> 6 hours post infection), viroplasms became refractory to the PG treatments, altering their liquid-like behaviour consistent with a liquid-to-gel/solid phase transition. Remarkably, PG treatments of cells during late infection stages also did not result in a significant drop of viral titres. To further investigate the dynamic composition of viroplasms, we have used reverse genetics approaches to rescue RVs harbouring a highly efficient biotin ligase (TurboID), fused to the viroplasmic scaffold protein NSP5 (NSP5-TurboID). We will use these tools to investigate the host proteins present in viroplasms using mass spectrometry-based proteomics methods. Identification of host proteins in viroplasms will be followed by either CRISPR or siRNA-based depletion of these proteins to assess the effects on RV replication and viroplasm formation.

B120

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

Infectious bronchitis virus (IBV) and infectious bursal disease virus (IBDV) are endemic in the UK and pose a threat to the productivity of the poultry industry, and to food security. Despite widespread use, vaccination-based control approaches face a number of challenges, e.g. rapid mutation and immune escape of the target virus. Since the replication cycles of IBV and IBDV are similar, as both viruses contain RNA genomes, enter through endocytosis, and replicate in the cytoplasm, the aim of this project is to identify common host genes that are involved in chicken-virus interactions through a genome-scale CRISPR/Cas9 knockout (GeCKO) screen. The chicken fibroblast cell-line DF-1 that has been demonstrated to support IBV and IBDV replication were suitable to conduct the screen. DF-1 cells were engineered to stably express Cas9, and the activity of the Cas9 was determined by a flow-cytometry based assay. DF-1 clones that had the highest activity were selected and propagated. A chicken lentivirus GeCKO library was generated and the screening strategy for cell-selection and sequencing were optimized. In addition, siRNA knockdown experiments have been optimized that will be performed in parallel to further identify potential pro-viral genes. We are now in a position to conduct GeCKO CRISPR and siRNA screens for IBV infection. The project will provide better understanding of IBV and IBDV replication, and the genes identified may potentially serve as candidates for generating virus-resistant chickens in the future.

B125

Generation of a true ORF10 knockout of SARS-CoV-2 allows direct analysis of the possible role of ORF10 in SARS-CoV-2 infection.

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Abstract

From the outset, the potential role of ORF10 has been difficult to elucidate. Only a small number of isolates have been found with mutations that abrogate the initiating methionine but there is an additional in-frame methionine which is maintained – thus most isolates maintain this ORF intact. Moreover, early studies examining ribosome translation initiation sites suggested that ORF10 should be expressed. Early streptavidin tagged ORF10 pull downs suggested a possible link to the ubiquitin ligase pathways but follow up studies could not find any functional effect on this pathway.

Independently we have utilised flag tagged ORF10 immunoprecipitation to show it may interact with MX1, an interferon induced antiviral host protein. In addition, we have used direct RNA sequencing and novel 5' cap labelling techniques to show that a small number of 5' capped ORF10 transcripts can be detected. We have previously used reverse genetics to generate a clone of SARS-CoV-2 based on the original strain isolated in Wuhan, China at the start of the pandemic. To further understand the role of ORF10 in virus replication we used site directed mutagenesis to remove two start codons within ORF10 and successfully rescued this clone. We also made clones of the virus replacing ORF10 with either the mNeonGreen or mScarlet fluorescent reporter proteins. We were unable to rescue fluorescent reporter protein viruses. Thus, we have tentative evidence that whilst ORF10 may be of utility, it is not essential for virus replication, but that there may be RNA structures needed within this region.

B126

Three species interactomic analysis of the MERS-CoV and SARS-CoV-2 envelope and membrane proteins

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Abstract

The Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are Betacoronaviruses capable of causing fatal human infections. Both viruses are believed to have emerged from bats *via* an intermediate host (camels for MERS-CoV, unknown for SARS-CoV-2) into the human population.

MERS-CoV and SARS-CoV-2 are enveloped positive-sense RNA viruses which encode four structural proteins (envelope (E), nucleocapsid, spike, and membrane (M)). The E and M proteins are involved in virus assembly, budding, envelope formation, and pathogenesis. Finding cellular protein interactors for these viral proteins, conserved across species, will increase our understanding of the coronavirus lifecycle and identify targets for antiviral development.

Three cell lines (human HEK293, bat *Pteropus alecto* PaKiT and *Camelus dromedarius* Dubca) were used for transient expression of the MERS-CoV and SARS-CoV-2 E and M proteins (FLAG epitope-tagged) followed by co-immunoprecipitation (co-IP) and high-throughput mass spectrometry-based interactomic analysis. Bioinformatic analysis revealed E/M protein interactions with ER, Golgi, mitochondrial and nuclear proteins. There were 32 high-confidence cellular interaction proteins conserved amongst the different cell lines and viruses ($p < 0.05$, 1.5-fold change compared to the controls). Initial validation of the results for 11 cellular proteins interacting with the MERS-CoV E and/or M proteins by co-IP/Western blot analysis and immunofluorescence co-localisation, confirmed 10 of the interactions. To determine the importance of these proteins and the 32 cellular proteins conserved across species, in the virus lifecycle, functional validation is currently being done by siRNA depletion in human cells, followed by infection with SARS-CoV-2/MERS-CoV.

B127

Investigation of SARS-CoV-2 Cases in UK Companion and Zoo Animals

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Abstract

The majority of SARS-CoV-2 infections during the pandemic have been restricted to humans. However, there have been numerous detections of the virus in other animal species, including cats, dogs, mink and large felids globally, as a result of reverse zoonotic transmission. Unfortunately, significant gaps remain in our understanding of the role of non-human species in the transmission of SARS-CoV-2, and the ability of animals to act as reservoirs and/or amplifying hosts. As such, the World Organisation for Animal Health (OIE) designated the detection of SARS-CoV-2 in animals as a reportable disease. The Animal and Plant Health Agency (APHA) is the National Reference Laboratory for SARS-CoV-2 in animals and has contributed to several disease investigations in both domestic and zoo animals. To date, APHA have identified SARS-CoV-2 in one cat, three dogs and three tigers, most of which were associated with known infected human contacts. Whilst the animals usually present with respiratory signs, other clinical disease involving the cardiovascular and haematopoietic systems have been observed but may be associated with underlying health conditions. However, asymptomatic infection can occur. Virological and molecular investigations were undertaken to identify the variant involved have been performed and will be presented. Investigating potentially infected animals thoroughly will further our understanding of the viral pathogenesis of SARS-CoV-2 in different animal species, as well as aid the definition of risk from different species in the transmission of SARS-CoV-2, and zoonotic threat. This data will also enable the improvement of countermeasures to prevent zoonotic and anthropozoonotic transmission events.

B128

Silencing of cellular entry proteins as therapeutic targets for SARS-CoV-2

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Abstract

SARS-CoV-2 enters respiratory epithelial cells through binding of its spike protein to the Angiotensin-converting enzyme 2 (ACE2) receptor after being primed by Transmembrane Serine Protease 2 (TMPRSS2).

Whilst several effective SARS-CoV-2 vaccines have been developed, there has been emergence of highly transmissible novel variants that have the potential for vaccine evasion. As such, we propose a novel gene therapy approach for targeting SARS-CoV-2 entry, by siRNA-mediated silencing of ACE2 and TMPRSS2. By targeting host proteins, we limit the possibility of evasion by SARS-CoV-2 variants.

We have developed air-liquid interface (ALI) cultures of normal human nasal epithelial (NHNE) cells that recapitulate human respiratory epithelial physiology with evidence of motile cilia and mucous secretion, plus lipid nanoparticles that effectively transfect these cells. Additionally, we have shown by qRT-PCR that following siRNA transfection we observe at least 80% knockdown of ACE2 and TMPRSS2 in submerged NHNE cells. This data is supported by western blot data demonstrating that siRNA knockdown of ACE2 leads to a reduction in protein levels.

We have generated SARS-CoV-2 spike pseudotyped lentiviruses that express GFP/luciferase that we will use to test the effect of this siRNA knockdown on viral entry. We will then confirm any effects observed with live SARS-CoV-2 to test the effect not only on entry but also viral propagation by RT-qPCR and plaque assays.

If successful, this therapy could provide protection against viral transmission as well as cell-to-cell dissemination.

B129

A measurement of longitudinal neutralising antibody response against pseudovirus spike-protein in serum from SARS-CoV-2 PCR positive patients

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Abstract

COVID-19 is a respiratory viral disease caused by SARS-CoV-2. Understanding how antibody responses evolve over time is vital in development of new therapeutics, diagnostics and vaccines. The purpose of this study was to measure the neutralising capacity of serum from SARS-CoV-2 positive patients (by PCR swab). This was achieved through a pseudovirus neutralisation assay to determine the ability of patient serum to neutralise SARS-CoV-2 spike protein interaction with HEK293T cells bearing the ACE-2 receptor and transmembrane serine protease 2 (TMPRSS2).

In total, 1,340 serum samples (anonymised excess diagnostic material) from 309 PCR positive SARS-CoV-2 patients were obtained sequentially (1-300 days post-PCR swab). This was part of the Development and Assessment of Rapid Testing for SARS-CoV-2 (DARTS) study undertaken at St. Georges Hospital NHS Foundation Trust. Serum were screened at 1/40 dilution, using a pseudovirus neutralisation assay. Neutralising serum (i.e. reducing pseudovirus transduction by >80%, pVNT80) underwent a further dilution series to determine the lowest titre that successfully reached a pVNT80.

Of the 1,340 serum samples screened at 1/40 dilution, >90% possessed neutralising antibodies to SARS-CoV-2 spike protein at pVNT80. Additional analysis includes a dilution series of neutralising serum, comparison of neutralising response between asymptomatic and symptomatic individuals as well as longitudinal antibody neutralisation.

We have demonstrated how the humoral immune response evolves over time in response to SARS-CoV-2 infection, with the vast majority of patients having neutralising antibody responses. Notably the data presented here are from a hospital cohort, so may not be representative of the wider population.

B130

Pseudotyped Bat Coronavirus RaTG13 is efficiently neutralised by convalescent sera from SARS-CoV-2 infected Patients

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Abstract

RaTG13 is a close relative of SARS-CoV-2, the virus responsible for the Coronavirus Disease 2019 (COVID-19) pandemic, sharing 96% sequence similarity at the genome-wide level. The spike receptor binding domain (RBD) of RaTG13 contains a large number of amino acid substitutions when compared to SARS-CoV-2, likely impacting affinity for the ACE2 receptor. Antigenic differences between the viruses are less well understood, especially whether RaTG13 spike can be efficiently neutralised by antibodies generated from infection with, or vaccination against, SARS-CoV-2. Using RaTG13 and SARS-CoV-2 pseudotypes we compared neutralisation using convalescent sera from previously infected patients as well as vaccinated healthcare workers. Surprisingly, our results revealed that RaTG13 was more efficiently neutralised than SARS-CoV-2. In addition, neutralisation assays using spike chimeras and mutants harbouring single amino acid substitutions within the RBD demonstrated that both spike proteins can tolerate multiple changes without dramatically reducing how efficiently they are neutralised. Moreover, introducing the 484K mutation into RaTG13 resulted in increased neutralisation, in contrast to the same mutation in SARS-CoV-2 (E484K). This is despite E484K having a well-documented role in immune evasion in variants of concern (VOC) such as B.1.351 (Beta). These results indicate that the immune-escape mutations found in SARS-CoV-2 VOCs might be driven by strong antibody pressures, and that the future spill-over of RaTG13 and/or related sarbecoviruses could be mitigated using current SARS-CoV-2-based vaccination strategies.

B131

Comparison of SARS-CoV-2 Vaccine-Induced T-cell Responses in Individuals with Immunodeficiencies and Healthcare Workers

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Abstract

We have recently reported on the poor/lack of early vaccine-induced antibody immune responses, approximately 32 days following the first dose of Covid-19 vaccination in both healthy individuals (healthcare workers) and those with various immune deficiencies. Here we provide T-cell immune response data in these same cohorts following first and second immunisation and analyse the relationship between T-cell response and immunodeficient patients' clinical profile.

Using direct ex-vivo Interferon-gamma ELISpot assay, SARS-CoV-2 spike-specific T-cell responses were assessed after the first and second (homologous) doses of the BNT162b2 or Vazxevria Covid-19 vaccines. We compared T-cell responses in immunodeficient patients (IDP) without prior SARS-CoV-2 infection (n=121) and healthy healthcare workers without prior infection (HCW_nPI) (n=55) or with prior infection (HCW_PI) (n=37). IDP had significantly lower number of positive responders compared to HCW_nPI although there was no significant difference in the magnitude of their responses. Not surprisingly, HCW_PI had significantly higher response. Interestingly, while there was no significant difference based on vaccine received within HCW_PI and HCW_nPI after completing the primary vaccination course, in IDP, recipients of Vaxzevria maintained significantly higher response after the first and second immunisations compared to BNT162b2 recipients. Analysis of T-cell responses based on IDP clinical profile showed a significantly lower response from IDP receiving immunoglobulin replacement therapy but no significant difference based on their diagnoses as having primary or secondary immunodeficiency. There was also no significant relationship between T-cell response and age of IDP. Further studies to elucidate the contribution of T-cell subtypes to overall immune response is ongoing.

B132

Inactivation of SARS-CoV-2 by Metal, Metal Oxide and Nanostructured Surfaces.

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Abstract

Human respiratory viruses can cause significant morbidity, mortality, economic-social disruption, exemplified by the SARS-CoV-2/COVID19 pandemic. Non-pharmacological interventions are determined by the mode of virus transmission; direct contact, fomite, droplets or aerosols. We investigate the antiviral activity of novel surfaces against SARS-CoV-2, with the aim of supressing fomite transmission. Metal, metal oxide and nanostructured surfaces were generated, exposed to SARS-CoV-2 (in DMEM/FBS) and inactivation determined by plaque assay. We confirmed the antiviral properties of copper: 100% SARS-CoV-2 inactivation occurred after 120 min copper exposure. We utilized the 50% inactivation exposure time of 30 min to screen a series of metal and metal oxide surfaces for antiviral activity. Copper oxide containing surfaces achieved significantly improved SARS-CoV-2 inactivation. Inactivation increased with increasing thickness of copper and copper oxide surfaces. Inactivation was also dependent on the SARS-CoV-2 carrier solution: copper exposed to SARS-CoV-2 resuspended in PBS was more efficiently inactivated than SARS-CoV-2 resuspended in DMEM/FBS. We hypothesized that virus inactivation is related to amount of metal ions dissolved into solution. However, ICP-MS revealed ~4-fold more copper ions are released into solution upon copper exposure to DMEM/FBS compared to PBS. Yet, DMEM dissolution fluid was not capable of inactivating virus, whereas PBS dissolution fluid resulted in ~50% virus inactivation. We also generated silicon nanostructured surfaces that inactivate SARS-CoV-2 to the same extent as copper surfaces. Our data suggest that multiple mechanisms result in virus inactivation on different surfaces and a better understanding is required for future rational design of optimal antiviral surfaces.

B133

Photocatalytic inactivation of enveloped and non-enveloped viruses including SARS-CoV-2 by titanium dioxide (TiO2)-containing plastic film

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Abstract

Infectious virions can persist on animate and inanimate surfaces for months if not removed. Such contaminated surfaces can contribute to disease transmission, particularly in high-risk settings such as healthcare environments. Here, we aimed to develop a novel, cost-effective, antimicrobial material for use in personal protective equipment (PPE) or on surfaces. To this end, we developed plastic films coated with titanium dioxide (TiO2), which when exposed to UVA light, photosensitizes the production of reactive oxygen species that degrade organic molecules rendering microbes, including virions, non-infectious. Influenza A virus (IAV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which are enveloped, and encephalomyocarditis virus (EMCV), which is non-enveloped, were used in this study under strict biosafety conditions. Virus inoculum (~10^6 $TCID_{50}$ in 50 µL) was added to TiO2-containing plastic films and incubated under UVA light for 0.25-3 hours, at a fixed distance. At each time point, the inoculum was harvested and residual infectivity was measured *via* titration. Plastic films lacking TiO2, as well as incubation in the dark, were used as negative controls. TiO2-film had potent virucidal activity against all viruses tested although differences in efficiency between viruses were noted. Furthermore, antiviral activity was dependent on UV incubation time and TiO2-concentration. We subsequently developed an improved TiO2-film which reduced virus titres from 10^8 $TCID_{50}$/mL to below the limit of detection within 2 hours. Given TiO2-containing plastics demonstrate antiviral activity, as well as being safe and affordable, we suggest such materials are attractive candidates for use in PPE or on surfaces in high-risk environments.

B134

The development of a SARS-CoV-2 replicon that can be maintained in cultured cells.

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Abstract

To develop more effective countermeasures against SARS-CoV-2 there is a need for technological platforms to rapidly assay SARS-CoV-2 replication in cell culture at containment level 2 (CL2), as rapid viral replication assays are hampered by the requirement to work at CL3. This study aims to develop a disabled biosafe form of the virus ("replicon") that can be used for replication studies at CL2.

SARS-CoV-2 replicons corresponding to the Wuhan-Hu-1 and Delta "variant of concern" viruses were generated by reverse genetics by "transformation-associated recombination" in yeast. The Spike and membrane genes were replaced with puromycin resistance and Renilla luciferase genes respectively. ORF6 or Orf7a were replaced with genes encoding the fluorescent proteins mScarlet or mNeonGreen. Additionally, the replicons contained mutations in Nsp1 encoding the amino acid substitutions K164A/H165A, predicted to decrease viral cytopathic effect. The cDNA clones were assembled in a YAC vector and preceded by a T7 RNA polymerase promoter. RNA transcripts produced from the clones by in vitro transcription were introduced into cells by electroporation.

The replicons replicate transiently in a variety of mammalian cell lines as evidenced by fluorescence, luciferase activity and immunostaining. Replication can be detected as early as 12 hours post-transfection (hpt); peaking at 36-48hpt. Transfection efficiency ranges from ~1% - 15% depending on the cell line used. Replicon replication has been found to be cytopathic and a range of cells are being screened to identify those which can be used to stably maintain replicon replication, to provide a non-infectious surrogate to study virus replication.

B135

SARS-CoV-2 variants of concern have extended mammalian ACE2 receptor host-range.

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Abstract

The continued evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the emergence of variants, some of which have been assigned as “variants of concern” (VOC). The Alpha, Delta and more recently, Omicron VOCs have all grown to global prevalence, displacing their predecessors. One characteristic of these variants are changes within the Spike protein, which have been demonstrated to have important implications for increased transmissibility, immune escape and modifying the host-range of the virus. SARS-CoV-2 is thought to have emerged from a bat reservoir, potentially through a yet undetermined intermediate host. Using a lentiviral-based pseudotyping system bearing the Spike glycoprotein, we have examined whether Alpha, Beta, Gamma, Delta and Omicron have differing patterns of mammalian ACE2 receptor usage, compared to the ancestral strain that emerged in Wuhan. All five VOCs were able to overcome previous restrictions for mouse ACE2, with VOC-specific differences seen for rat, ferret, civet or bat receptors. We were also able to attribute these changes in host range to substitutions within the RBD, namely N501Y and E484K. Understanding the host-range of SARS-CoV-2 can inform risk to animals, their capacity to serve as viral reservoirs, and to establish and validate animal models.

B136

Bi-cyclic peptides (Bicycles®) targeting ACE2, a novel therapeutic modality for SARS-CoV-2

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Abstract

SARS-CoV-2 is being tackled via a comprehensive worldwide vaccination program, but the virus is likely to become endemic and continue to evolve driving multiple new waves of infection. Complementary therapies are currently based on the use of convalescent plasma or monoclonal antibody cocktails, which have high production costs, poor pharmacokinetics (PK) and require delivery via intravenous administration, or small molecule anti-viral drugs with limited therapeutic utility. Here, we describe our screening efforts to identify and optimise a third class of potential SARS-CoV-2 therapeutics, namely bi-cyclic peptides (*Bicycles*). *Bicycles* are synthetic constrained peptides which, as small molecules, have good drug-like properties, can be produced at scale, have attractive PK properties (instantaneously distributing to extracellular fluid), a limited requirement for cold chain and can be administered by a range of convenient routes, including intra-nasal and subcutaneous. Uniquely, *Bicycles* binding ACE2 can be combined in a single molecule to gain affinity and avidity advantages and demonstrate encouraging anti-viral activity *in vitro*. Here, we will describe the identification of *Bicycles* targeting ACE2, their optimisation to high potency molecules with drug-like properties and their effectiveness in *in vitro systems*.

B137

Survival of SARS-CoV-2 virus variants on Skin and Skin-to-Skin Transference

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Abstract

SARS-CoV-2 is a major global health issue and whilst vast amounts of research has been conducted to characterise this virus, substantial gaps remain. SARS-CoV-2 has been shown to survive on skin and surfaces, but variability among virus variants, as well as multiple skin-to-skin touch contacts on viral survival and transference is unknown. This is critical in understanding how SARS-CoV-2 expelled from an infected person results in contamination of their environment and can potentially initiate onward infection(s). To address this, we have utilised porcine skin as a surrogate of human skin to assess survival of SARS-CoV-2 variants (Alpha, Beta, Gamma and Delta) on skin and the transference of virus through skin-skin contacts. Firstly, it was found that all SARS-CoV-2 variants were detectable by real-time RT-PCR after 72 hours at biologically-relevant temperatures (35.2°C), but, the quantity of viral RNA was reduced when on porcine skin compared to a plastic surface. Secondly, SARS-CoV-2 variant viruses were applied to porcine skin as either a droplet or a bioaerosol and the number of transferences assessed after multiple skin-skin contacts. Interestingly, regardless of application method, viral RNA was detected after four successive skin-skin contacts. This work shows that SARS-CoV-2 variants can persist on skin and be transferred through skin-skin contacts, highlighting a potential risk pathway for SARS-CoV-2 infection, with relevance to both public and veterinary health. Work is ongoing to assess whether SARS-CoV-2 on skin is capable of establishing infection *in vivo*, which will further our understanding of SARS-CoV-2 risk pathways.

B138

Insight into host responses in COVID-19 and Influenza patients at point of care and in a mice model using RNA sequencing

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Abstract

Rapid investigations throughout the pandemic has facilitated realtime genome surveillance and characterisation of disease, influencing public health responses and improvement of patient care. Both Influenza A and SARS-CoV-2 infections can result in severe disease and hospitalisation, therefore whole blood samples were collected from IAV patients and COVID-19 patients in the first wave and prior to the availability of medical countermeasures, allowing insight into a wildtype SARS-CoV-2 infection. RNA from COVID-19 patients (n = 82), Influenza patients (n=88) and healthy controls (n=5) were sequenced on the Illumina Novaseq and a subset was sequenced on the GridION. Through differential gene expression analysis, a plethora of transcripts were identified that were unique to COVID-19 vs IAV, or fatal COVID-19 vs non-fatal COVID-19. Through both Illumina and nanopore sequencing, immunoglobulin transcripts were identified at an increased abundance when comparing COVID-19 and IAV patient transcriptomes. Likewise, when comparing fatal to non-fatal COVID-19. Transcripts associated with the adaptive immune response were in lower abundance in individuals with fatal COVID-19 in comparison to those who survived, suggesting a delayed adaptive response could be associated with fatality. Although nanopore sequencing identified less transcripts than illumina sequencing, a strong correlation between datasets was observed, highlighting the use of nanopore for rapid elucidation of host responses. The transcripts observed in humans were compared to those in a mouse model of severe infection. Highlighting common genes that were essential in the host response. Together with post-mortem analysis of diseased tissue this paints a picture of uncontrolled immune pathology.

B139

Characterisation at the organ level of SARS-CoV-2-induced macrophage-dependent inflammation in the spleen

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Abstract

Despite an intense research effort aspects of the pathogenesis of the novel coronavirus SARS-CoV-2 remain unclear. A feature of many severely ill COVID-19 patients is septic shock, however we have little understanding of the events leading to COVID-19 sepsis. The observation that SARS-CoV-2 survives in $CD169^+$ cells within the spleen[1,2] leads to the suggestion that sepsis originates from a reservoir in the spleen and is consistent with inflammasome activation by SARS-CoV-2. This picture is reminiscent of our previous findings on how some bacterial pathogens cause sepsis[3,4].

We have ethical approval to use human spleens, from partial pancreatectomy, for the study of infection in *ex vivo* organ perfusion (Clinical Trials NCT04620824, REC18/EM/0057). As cell behaviour and marker expression may change during primary cell culture, the ability to infect intact human organs presents a unique opportunity to study early SARS-CoV-2 events. Post surgery, spleens are cannulated and flushed with preservative solution before transport to the CL3 laboratory on ice. The organs are washed with heparin-saline solution, before connection to an Organ Assist kidney-perfusion apparatus (XVIVO) for perfusion with Oxyglobin perfusate. Normothermic perfusion has been performed for up to 8 hours.

Perfused spleens are infected with 10^7 SARS-CoV-2 virions and perfusate samples and tissue biopsies are taken at intervals up to 8 hours. Antibody staining of viral proteins and cell-specific markers combined with high-content fluorescence scanning microscopy allows placement of cellular or tissue infection events into the context of the spleen microanatomy.

[1]Feng 2020 doi.org/10.1101/2020.03.27.20045427

[2]Park 2020 doi.org/10.1038/s41577-020-0317-2

[3]Ercoli 2018 doi.org/10.1038/s41564-018-0147-1

[4]Chung 2018 doi:10.14573/altex.1805131

B140

The SARS-CoV-2 Spike Protein Mutation Explorer: Using Visualisations and Interactivity for Improved Communication about Variants of Concern

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Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the cause of an ongoing pandemic with severe impacts worldwide. The SARS-CoV-2 spike protein, which is primarily involved in viral infectivity, transmissibility, and antigenicity, is rapidly mutating. As mutations accumulate, Variants of Concern (VOCs) emerge, increasing the pandemic's severity. Throughout the pandemic, the COVID-19 Genomics UK consortium (COG-UK) has used SARS-CoV-2 sequence data to share details about VOCs with expert audiences via their Mutation Explorer (ME). It's also important for VOC information to be made highly accessible to a general audience, who lack expertise in molecular virology but whose daily lives will be directly influenced by mutations in the SARS-CoV-2 spike protein. In molecular biology education, visualisations and interactive applications have been shown to improve understanding of protein structure and function, and to boost engagement and active learning. Therefore, we used these methods to create the SARS-CoV-2 Spike Protein Mutation Explorer (SSPME), an educational, interactive visual resource for the COG-UK/ME. This application places the mutations characteristic of VOCs in the context needed by non-specialists, using an interactive spike protein 3D model, narrated animations depicting SARS-CoV-2 entry and neutralisation, and glossaries of relevant terms. User-testing, on participants recruited through social media, showed that compared to the specialist data presentation of COG-UK/ME, the SSPME offered substantially better usability, and significantly improved knowledge acquisition and confidence. Our results show that the SSPME provided an improved platform for communicating complex, topical information about SARS-CoV-2 spike protein mutations and VOCs to the wider public.

B142

Analysis of SARS-CoV-2 in nasopharyngeal samples from patients with COVID-19 illustrates population variation and diverse phenotypes, placing the growth properties of Variants of Concern in context with other circulating lineages.

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Abstract

New variants of SARS-CoV-2 are continuing to emerge and dominate the global sequence landscapes. Several variants have been labelled as Variants of Concern (VOCs) because of perceptions or evidence that these may have a transmission advantage, increased risk of morbidity and/or mortality or immune evasion in the context of prior infection or vaccination. Placing the VOCs in context and the underlying variability of SARS-CoV-2 is essential in understanding virus evolution and selection pressures and identifying whether a particular lineage is truly a VOC. Consensus sequences and the population genetics of SARS-CoV-2 in nasopharyngeal swabs from hospitalised patients were characterised. Changes at a minor variant level were identified. Variants with the nsp12 P323L variation also contained minor variants at that position including P and F and other amino-acids. To place the Alpha, Beta and Delta VOCs in context their growth was compared to a selection of other clinical isolates of differing lineages from different times during the pandemic. The data indicated that the growth in cell culture of Alpha was less than the other VOCs in human cells, suggesting that its apparent transmission advantage was not down to replicating more quickly. Growth of the Beta and Delta variant were towards the higher end of the variants. Overall, the study suggested that studying the biology of SARS-CoV-2 is complicated by population dynamics and that these need to be considered with new variants. In the context of variation seen in other coronaviruses, the variants currently observed for SARS-CoV-2 are very similar.

B143

EMoMiS Tool Identifies Molecular Mimicry Between SARS-CoV-2 Spike and Many Viral Immunogenic Proteins

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Abstract

Epitope-based molecular mimicry occurs when an antibody cross-reacts with two different antigens because of their structural and chemical similarities. Molecular mimicry between proteins from two different pathogens can lead to beneficial cross-protection when an antibody produced by exposure to one is able to react with the other, thus possibly facilitating the repurposing of vaccines. There are no computational tools available to date for a large-scale search of antibody cross-reactivity. We present a comprehensive Epitope-based Molecular Mimicry Search (EMoMiS) pipeline to address this need. As a first step in the EMoMiS pipeline, antigens extracted from the Structural Antibody Database (SAbDab) are searched for regions of sequence and structural similarity with the target protein. Then, a pre-trained deep learning model is used to evaluate if antibodies, known to recognize the SAbDab antigens, can cross-react with the target structure. When we applied EMoMiS to the SARS-CoV-2 Spike protein, mimicry epitopes from the Dengue virus, HPV-16, HIV-1, and coronavirus OC43 viral proteins were identified. Possible antibody cross-protection caused by exposure to those viruses may explain the differential severity of COVID-19. The developed pipeline is taxonomically agnostic and can be used in the early stages of viral outbreaks in predicting the immune response induced by molecular mimicry. It can also be useful in designing broad-spectrum vaccines and vaccine targets. Note that for vaccine repurposing, the binding affinity of the cross-reacting antibodies may be lower. This is addressed by EMoMiS by predicting the strength of binding.

B144

A longitudinal study sampling train and bus surfaces across England for SARS-CoV-2 RNA contamination

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Abstract

Public transport is used by most demographics and is an essential mode of travel. The risk of respiratory virus acquisition on public transport is unknown.

As part of the ESPRC funded TRACK project, 48 surface samples were taken from three bus operators and 40 samples from two train operators, before cleaning, across different areas of England. The samples were cultured for bacterial enumeration on Columbia blood agar. Following RNA extraction, quantitative RT-PCR was used to detect SARS-CoV-2 RNA.

The percentage of samples taken from buses and trains with detectable SARS-CoV-2 RNA ranged from 0-50% and 5-45% respectively, with higher overall detection in January/February and July/August 2021. SARS-CoV-2 RNA was detected on 22% of train air vents (n=80) and tables (n=40), on ~10% of bus handholds (n=144) and window hoppers (n=61) and ~10% of seat head rests (n=234) from trains and buses, among other surfaces. RNA levels were consistently lower than 560 copies per extraction, making the presence of live virus at the time of sampling highly unlikely. As air vents are not routinely cleaned, the presence of viral RNA could be due to accumulation of historical contamination. Bacteria, including some associated with oral and nasal cavities, were isolated from all areas.

Aside from air vents, SARS-CoV-2 RNA was more frequently detected on touch points requiring a forceful grabbing motion and/or prolonged contact compared to those requiring a light touch like open buttons. These results will inform modelling and ongoing mitigation strategies to ensure public transport remains safe.

B149

Air virome composition in farm buildings across different degrees of urbanization

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Abstract

The microbiome of the built environment (BE) comprises bacteria, fungi and viruses that live in the air and on surfaces within human-built structures. Viruses in the BE were of interest prior to the outbreak of the COVID-19 pandemic, especially their distribution in our homes and seasonal patterns. However, due to the difficulty of their recovery and analysis, viruses are often omitted from microbiome studies in the BE. This project aims to characterize the viral population of the air microbiome of two farms over time and determine the impact of urbanization and building design on virome composition.

Air samples were collected from a city and a rural dairy farm once a month for six months using a passive sampling approach. Metadata was gathered in parallel according to minimum standards guidelines (MIxS-air, MIxS-BE) which allows for an in-depth study of the impact of environmental and building conditions on the BE microbiome (temperature, humidity, occupancy...). Viral RNA and DNA were co-extracted from air samples and subjected to whole genome sequencing. A custom bioinformatics pipeline using the NCBI viral database was created to assign taxonomy and abundance to the sequences. Diversity analyses were performed in association with the metadata, including building characteristics and weather conditions, to determine the virome response in the two farms.

Preliminary results will be presented, providing groundwork on the BE virome that will be used to perform future functional analyses of the microbiome in rural and urban environments, as well as to re-think urban design to benefit human health.

B151

Identification and characterisation of Carbohydrate Active enZymes in Z*obellia galactanivorans* DsijT using quantitative proteomics

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Abstract

Zobellia galactanivorans Dsij^{T} is a marine flavobacterium, first isolated from red algae, that has become a model organism for algal polysaccharide degradation. Previous studies have analysed the complete genome, and transcriptome-wide gene expression. These studies have identified many Carbohydrate Active enZymes (CAZymes), including glycoside hydrolases, polysaccharide lyases, carbohydrate esterases, and sulfatases. Many of these CAZymes are organised into polysaccharide utilisation loci (PULs). Despite previous studies into the enzymes involved with polysaccharide degradation by *Z. galactanivorans*, many of the identified PULs contain proteins of unknown function. The aim of this work was to identify and characterise some of these proteins of unknown function. To identify proteins involved with specific polysaccharide degradation we carried out quantitative proteomic analysis of the secretome. Proteins identified in these studies as being expressed in significantly higher amounts when grown on a specific polysaccharide compared to a glucose control, were selected for recombinant expression. Current work is involved with characterising these proteins for their function and role in polysaccharide degradation within *Z. galactanivorans*.

B152

Microplastics As Vectors of Antimicrobial Resistance in Aquatic Systems

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Abstract

Antimicrobial resistance (AMR) is a significant global threat to human health, with the environment playing an increasingly important role in the evolution and spread of AMR. Microplastics are ubiquitous in aquatic systems and regularly co-occur with AMR bacteria, human pathogens, and antimicrobial residues in faecal contaminated environments. Microplastic associated biofilms, referred to as the 'Plastisphere', have been found to enrich AMR bacteria and can be distinct from the surrounding environment and natural debris. This research will investigate and compare biofilm and free-living bacterial communities following a lab-based sewage incubation with environmentally aged microplastics and natural or inert substrate controls. Using both culture- and molecular-based methods, the data generated by this research could enable a greater understanding of the potential selective colonisation of pathogenic and antibiotic resistant faecal indicator organisms on microplastic surfaces. Given that microplastics are extremely prevalent and persistent, this may have significant consequences on human and environmental health, including increasing human exposure to drug-resistant pathogens in coastal environments. Therefore, the outcomes of this research may help to inform waste management practices and environmental monitoring of both microplastics and AMR.

B153

BACTERIAL COMMUNITIES IN THE BELOWGROUND COMPARTMENTS OF POTATO PLANTS

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Abstract

Bacterial communities of the potato (*Solanum tuberosum*) rhizosphere have been studied in detail as part of the aim to improve plant productivity. However, less is known about the tuberosphere (soil attached to the tuber surface) bacteria and how they compare to the structure of bulk soil and rhizosphere. A paucity of recent studies indicated that the tuberosphere may select for a distinct bacterial community.

Bacterial tuberosphere, rhizosphere and bulk soil communities were analysed in potato varieties Kerr's Pink and Rooster grown commercially in two fields with different physicochemical characteristics. Illumina sequencing of 16S rRNA gene libraries revealed a similar diversity (Shannon index) and richness (Chao1) between the three compartments of both varieties, indicating that alpha diversity was not affected by the soil compartment. Non-metric Multidimensional Scaling demonstrated that bulk soil and tuberosphere communities clustered together and were different from the communities in the rhizosphere. These results were largely confirmed by ANOSIM testing. Furthermore, relative abundance analysis revealed that Proteobacteria, Firmicutes, Actinobacteriota and Acidobacteriota were the most abundant phyla and presented differences in the three soil compartments of both varieties. Soil compartments had also a significant effect on arylsulfatase activity that was highest in the rhizosphere in both varieties. Likewise, the abundance of sulfonate utilizing bacteria was also highest in the rhizosphere.

Different mechanisms appear to be involved in the assembly of bacterial communities in the rhizosphere and tuberosphere with the latter being highly similar to the bulk soil communities, putatively due to a lack of exudates stemming from the tuber.

B154

The Potential for Phosphite Oxidation in Bacteria and Archaea

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Abstract

Microbial phosphorus (P) metabolism has long thought to centre around phosphate, while the potential for P redox transformations has largely been ignored. However, the discovery of bacteria able to oxidize phosphite into phosphate has begun to reshape our understanding of the biogeochemical P cycles of the contemporary and early Earth. Yet only a handful of phosphite oxidising organisms have been identified thus far.

A search for homologs of phosphite dehydrogenase (PtxD, the major enzyme responsible for phosphite oxidation) revealed 7507 proteins, which were then used to generate a sequence similarity network. One cluster of 514 sequences shared at least 48 % sequence similarity with functionally characterised PtxD sequences and, thus, this cluster was thought most likely to contain functional PtxD enzymes. The genome neighbourhoods of the sequences in this cluster were visualised and compared to those of characterised PtxD sequences. Highly conserved gene neighbourhoods were found with over 300 of these sequences. Therefore, the organisms containing these conserved gene neighbourhoods are promising novel candidates for microbial phosphite oxidation, the discovery of which would aid in the continuing characterisation of this ill-defined metabolism. Additionally, the candidate PtxD sequences were screened for protein motifs and several *de novo* motifs were identified, providing a possible tool to screen and identify more novel PtxD enzymes. These findings might explain the disconnect between the apparent prevalence of PtxD and the dearth of known phosphite oxidising organisms, while helping to characterise and identify novel phosphite oxidising organisms.

B156

Filamentous Fungi as an environmental alternative to traditional food

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Abstract

As we start to switch to new food sources as a way to tackle climate change, it is important we understand the physiological effects they will have on our metabolic systems to ensure any changes made are safe and aim to improve our overall health where possible. Changes in diet, such as switching to meat alternatives like Quorn, affect the population of different microbial species within the human gut microbiota (HGM) which previous research has indicated contributes to the wellness of the host organism, as an unhealthy HGM can lead to severe diseases both of the gut and of the brain via the gut-brain axis. Quorn chiefly consists of mycoprotein obtained from Fusarium venenatum, it is partly indigestible due to the fibres in its cell wall, one of the fibres in mycoprotein is a complex glycan called mannoprotein. These fibres rely on bacterial fermenters within the HGM to digest them into short-chain fatty acids (SCFAs) which are obtainable by the host. Different fermenters produce different SCFAs, and imbalances in SCFAs can lead to severe disease formation due to the relationship they have with mucous formation in the intestine. It also means through the addition of certain food sources imbalances can be corrected (prebiotic). This project is seeking to find out if mannoproteins from F. venenatum and other species of fungi are capable of sustaining a healthy and diverse HGM that is beneficial to the host, and if it can be used as an effective prebiotic.

B157

Characterising the proteomic response of mushroom pathogen *Lecanicillium fungicola* to *Bacillus velezensis* QST 713 and Kos

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Abstract

Infection by the fungal pathogen *Lecanicillium fungicola* produces dry bubble disease in *Agaricus bisporus* cultivation and diseased mushrooms significantly reduce yield and revenue for mushroom growers. *L. fungicola* strains are emerging which are resistant to chemical fungicides. These fungicides are being phased out because they can be harmful to the environment. Biocontrol agents may represent an alternative and more environmentally friendly treatment option to help control dry bubble. SERENADE® (AgraQuest Inc.) is a commercially available biocontrol product which has been used to treat plant diseases. We investigated the *in-vitro* response of *L. fungicola* when exposed to the bacterial strain active in SERENADE®, *Bacillus velezensis* (QST 713). We also investigated its response to a newly isolated *B. velezensis* strain (Kos) to analyse its potential as a novel biocontrol strain. *B. velezensis* (QST713 and Kos) showed inhibition against *L. fungicola* in plate and liquid cultures. The proteomic response of the pathogen against these biocontrol strains was also investigated. Proteins involved is growth and translation such as 60S ribosomal protein L21-A (-32-fold) and 40S ribosomal protein S30 (-17-fold) were reduced in *B. velezensis* QST 713 treated samples, while proteins involved in a stress response were increased (Norsolorinic acid reductase B (47-fold) and (isocitrate lyase (11-fold). *L. fungicola* was found to have a similar proteomic response when exposed to *B. velezensis* (Kos). This work provides information on the response of *L. fungicola* to SERENADE® and indicates the potential of *B. velezensis* Kos as a new biocontrol option for controlling fungal diseases of mushrooms.

B158

Investigating the role of an orphan response regulator in the MtrAB signal transduction system

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Abstract

Streptomyces are gram-positive, filamentous bacteria that produce a variety of secondary metabolites (SM), including 55% of antibiotics used today. Often, SMs are encoded by locally clustered genes participating in a specific metabolic pathway, otherwise known as biosynthetic gene clusters (BGCs). Genome sequencing has revealed ~90% of BGCs in Streptomyces spp. are not expressed under laboratory conditions, meaning many SMs, including antibiotics, are awaiting discovery. A better understanding of the regulatory pathways controlling antibiotic expression is required to 'unlock' these cryptic BGCs and potentially unveil novel antimicrobials, which are in demand due to the increasing threat of antimicrobial resistance.

This project focuses on MtrAB, a highly conserved two-component signalling system that coordinates antibiotic production with sporulation in Streptomyces venezuelae. MtrA is a transcriptional regulator controlled via phosphorylation and dephosphorylation by sensor kinase, MtrB. MtrA is an essential master regulator that heavily contributes toward secondary metabolism in Streptomyces spp. For example, it directly regulates chloramphenicol repression in S. venezuelae and controls the production of antibiotics, actinorhodin, and undecylprodigiosin, in Streptomyces ceolciolor. Also, MtrA binds to 85% of BGCs in S. venezuelae. Therefore, MtrA is likely an important component in unlocking cryptic BGC expression.

MtrA controls the expression of more than 500 genes in S. venezuelae but these genes do not contain a conserved MtrA consensus binding sequence. In this work, we investigate if and how MtrA interacts with other regulatory proteins to control the expression of different subsets of target genes to further understand MtrAB and its influence on antibiotic production

B160

Antimicrobial resistance genes in urban sewage show evidence of universal dynamics: a global study

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Abstract

Evaluating how dispersal and selection influence distributions of AMR genes around the world can inform best public health management practices for limiting the risks of hazardous AMR genes. We studied the universality of AMR genes found in sewage sites from around the world to explore dispersal and selection influences. A universal system has the same selection pressures at each site, with differences in local microbiomes and AMR genes caused by different colonising bacteria.

The Global Sewage Surveillance Project (GSSP) provided AMR genes from 79 sites in 60 countries around the world. With this data, the overlap (number of shared species in a pair of samples) and dissimilarity (root Jenson-Shannon divergence distance in a pair of samples) of AMR genes was evaluated using multiple-membership mixed effects models.

Dissimilarity and overlap of AMR gene compositions in the GSSP data had a negative relationship, as expected in a universal system. This universality was still found when antimicrobial usage practices, socio-economic factors and geographic separation were considered. Our results indicate dispersal and dispersal barriers are critical factors for establishing global AMR gene distributions. Preventing dispersal of newly emerged AMR genes could severely hamper their global spread.

B161

Effects of surface roughness on anaerobic marine biofilm formation and microbiologically-influenced corrosion of UNS G10180 carbon steel

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Abstract

The challenge in understanding and predicting microbiologically influenced corrosion (MIC) is the lack of robust and reproducible model biofilm systems that reflect real-world operating conditions. Furthermore, there are no internationally recognised standards or test methods with which to evaluate control strategies effective against MIC. Current industrial standards provide insightful guidance when it comes to the detection, testing and evaluation of MIC; however, less than 25% of risk-based inspections analyse sessile biofilm samples when investigating corrosion.

This work aims to develop and validate a model biofilm system to investigate the role of biofilm communities within MIC. The effect of surface roughness on MIC and biofilm formation between As Received, and 25 µm polished carbon steel coupons (UNS G10180) will be investigated. The objective is to run two CDC biofilm reactors, one control and one test reactor inoculated with an anaerobic marine sediment sample. Both reactors will be run with an electrochemical cell setup and H_2S microsensor, whilst maintaining anaerobic conditions.

Corrosion rates will be monitored daily via linear polarization resistance and electrochemical impedance spectroscopy measurements, with potentiodynamic polarization performed at the end. Similarly, changes in H_2S concentration will be monitored daily. Once the experiment is complete, biofilm viability through LIVE/DEAD imaging and monitoring of ATP activity will be assessed. Gravimetric analysis alongside surface profilometry will be performed to assess the extent of the corrosion degradation.

We hypothesise that carbon steel coupons with greater surface roughness will facilitate biofilm attachment and growth, and thus exhibit higher corrosion rates.

B162

Engineering a native *Kluyveromyces marxianus* transporter to reprogramme the balance between hexose and pentose uptake

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Abstract

Sustainable and efficient use of lignocellulosic hydrolysates in yeast-based biorefineries requires the simultaneous consumption of both the hexose and pentose sugars present in this feedstock. This represents a key bottleneck because of slow uptake of pentoses and the preferential use of glucose by most yeasts. To identify novel pentose transporters that could be a resource for transporter engineering, we developed a pentose transporter screening platform in the pentose utilising yeast *K. marxianus*. Twelve genes encoding transporters of the major facilitator superfamily (MFS) were deleted to create a host unable to grow on pentoses. Candidate transporters were then heterologously expressed and strains screened for growth on pentose sugars. Several *K. marxianus* proteins that could transport xylose and arabinose were identified and kinetic studies performed on the most promising candidates using ^{14}C-labelled sugars. One of these transporters showed high affinity for both xylose and glucose, though the affinity for glucose was still 150-fold higher than for xylose. Using *in silico* analysis we selected target amino acids of this potential transporter for site-directed mutagenesis in an attempt to convert it into a specific xylose transporter. Several mutants were constructed and characterized by radioactive uptake and growth assays in bioreactors on mixture of sugars. One of these mutants lost the ability to transport glucose but still transported xylose with high affinity kinetics. This successful reprogramming of the transporter to increase its preference for xylose over glucose was used to facilitate efficient co-consumption of hexose and pentose sugars in an engineered *K. marxianus* strain.

B163

Up-scaling poly(γ-glutamic acid) (γ-PGA) biosynthesis

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Abstract

Poly(γ-glutamic acid) (γ-PGA) is an anionic, and biodegradable, pseudopolypeptide extracellularly synthesised by several *Bacillus* species. γ-PGA can present diverse characteristics based on the ratio of D, L or D/L acid residues in the polymeric chain, the molecular mass (Mw) and its form (free acid form or salt form). This variety in material's properties enables it to satisfy an array of sector-specific applications, ranging from bioremediation to medical materials. However, biosynthesis of this material is still not reproducible nor cost competitive.

The aim of this study was to up-scale the production of this material whilst maintaining it's reproducibility. All small-scale shake-flasks experiments (250 mL volume) were performed under aerobic conditions (150 rpm). Medium scale experiments (4 L volume) were performed under aerobic conditions (250 rpm, 2 L/min). *Bacillus subtilis* natto ATCC 15245 cells were grown at 37°C for 96hrs. To monitor microbial growth, samples were withdrawn at 24hrs intervals. Samples were serially diluted, plated on TSA and incubated overnight at 37°C. Ultimately, cell numbers were counted and results expressed as CFU ml^{-1}.

We investigated the effects of medium components (glutamic acid, sugars and algal fraction) on poly(γ-glutamic acid) yields and microbial growth (CFU ml^{-1}). Further, the material was characterised with focus on its molecular mass, D/L enantiomeric ratio, mineral content and bond type. Overall, the growth rates were comparable, however productivity of γ-PGA was significantly higher within 4 L fermenter compared to shake flasks.

B164

Portable HEPA filtration successfully augments natural ventilation-mediated airborne particle clearance in a legacy design hospital ward

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Abstract

The COVID-19 pandemic has galvanised research into airborne disease transmission, leading to widespread acceptance of SARS-CoV-2 transmission by airborne particles, particularly in indoor poorly-ventilated environments. This has implications for hospital design, and immediate relevance for legacy hospitals in Ireland and the UK lacking mechanical ventilation in most clinical areas. Portable HEPA filtration has been shown to remove SARS-CoV-2 RNA in air samples from COVID-19 surge hospital units. We report the effect of portable HEPA filtration in clearing a common hospital air pollutant (nebulised salbutamol) from a ward bay under renovation.

Methods: 2.5 mg Salbutamol was nebulised 4 times (Window open filter on, filter only, window only, window closed filter off) in a 171 m3 ward bay with three top-hinged windows facing 169° (SSE), windspeed 2.6-5.1 m/s Westerly, entrance door sealed with a polythene barrier. Airborne particulate matter (PM2.5) was monitored at five locations with individual AirVisual Airnode (IQAir, China) laser sensors. The CC2000 HEPA filtration device (Camfil-Ireland) was operated at 760 m3/hr.

Results: Mean peak particulate matter (PM2.5) was lowest with open windows and filter operation, less than 50% of the next lowest condition of filtration only. Highest variability of PM2.5 between monitors was seen with windows open without filtration. Mean PM2.5 clearance rate was significantly higher with open windows and filtration than filtration alone, in turn significantly higher than windows alone.

Conclusions:

Portable HEPA filtration successfully augmented natural ventilation in airborne particle clearance from a legacy design hospital ward, both by increasing clearance rate and reducing spatial variability.

B166

Metagenomic and Physico-Chemical Characterisation of a Novel Microbial Biofilm in a Deep Ground Water Well in Ireland

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Abstract

Biofilms consist of a variety of molecular compounds, such as extracellular DNA, polysaccharides, lipids, proteins, including microorganisms, which are either beneficial or harmful to human health. The main issues involving biofilms include blockages in pipelines, erosion of surfaces and, emission of foul odours, such as Hydrogen Sulfide (H2S). The biofilm that I am currently investigating came from a groundwater well on a farm in Loughnavalley, County Westmeath. The objective of the project is to identify the microbes responsible for producing this biofilm, which is preventing the farmer to utilize the water source on his dairy farm, and find a solution to disrupt its formation. Sulfur is a key element responsible for developing biofilms within aquatic environments, which can be found in ecosystems and exists in bedrock, as either pyrite (FeS2), a dangerous mineral containing sulfur gas, which can contaminate groundwater systems, or gypsum (Ca2SO4), a sulfate mineral that can uptake water and reduce soil erosion upon the surface of the Earth's crust.

This project focuses on (1) the chemical nature of the EPS material, (2) the genes involved in the production of the EPS (through whole-genome sequencing of microbial isolates), and (3) the environmental conditions necessary (pH, redox potential, nutrient concentrations, etc) for the formation of the EPS.

B167

Enrichment and selection of pharmaceutical-tolerant electroactive microbial communities

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Abstract

We studied the enrichment and acclimatization of natural microbial communities in microbial electrochemical systems (MES) and non-MES reactors in the presence of the biologically active pollutants atenolol (ATL), carbamazepine (CAR), and ketoprofen (KET). We used 16S rRNA gene amplicon sequencing to analyse the enriched microbial populations at the phylum, class, and genus level. Although the media composition and environmental conditions were similar, there was a significant difference between the structure and composition of anodic microbial communities and the cultures enriched in non-MESs cultures. The Shannon index for anodic biofilms of MESs was higher than in non-MESs cultures receiving the same pharmaceuticals, indicating that higher diversity is achieved in an electrochemically active system. The analysis at the genus level showed that the anodic biofilms of ATL-MES and CAR-MES were dominated by *Geobacter* (21.5 % and 33.8 %, respectively), while in KET-MES, *Pseudomonas* had the highest relative abundance (55.9%). In contrast, the microbial communities in ATL-non-MES, KET-non-MES, and CAR-non-MES were dominated by *Methanobrevibacter* (54.47%, 65.15%, and 65.29% respectively). The electrochemical performances of the anodic biofilms confirmed the successful enrichment of electroactive bacteria, with maximum current densities of 215.64 (ATL), 272.01 (CAR), and 465.46 mA/m2 (KET) were obtained. The negligible relative abundances of methanogens in MES biofilms demonstrate that electroactive bacteria outcompeted methanogens. The results show that strategies for selecting pharmaceutical-tolerant electroactive microbial communities can be used in the design of MES for wastewater treatment.

B168

Developing an xMAP multiplex assay for the rapid and simultaneous detection of AHSV serotypes 1-9

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Abstract

African horse sickness (AHS) is an arthropod-borne disease of equids that is often fatal in naïve horses and mules. AHS is currently endemic in most of sub-Saharan Africa but outbreaks have occurred in India, the Middle East, Spain and Thailand. The causative agent is the African Horse Sickness Virus (AHSV), genus *Orbivirus*, for which there are nine antigenically distinct serotypes. In order to facilitate surveillance programmes and deploy appropriate control strategies where outbreaks occur, rapid, accurate and cost-effective diagnostics are essential. Over the past decade, Real Time-PCR assays have been developed that allow for differentiation of AHSV based on serotype, however when the serotype is unknown these assays can be costly and time consuming. xMAP technology allows for simultaneous detection of multiple targets and has previously been used to detect all 24 serotypes of Bluetongue Virus, also part of the *Orbivirus* genus, proving itself to be a useful diagnostic tool when analysing large sample sets. xMAP technology works by detecting fluorescent beads that are hybridised with amplified nucleic acid specific to each AHSV serotype, if it is present in the sample. Due to the multiplexing nature of the xMAP platform, the cost and time involved in the analysis of samples can be greatly reduced. Here we describe the development and optimisation of an xMAP multiplex assay for the simultaneous detection of AHSV serotypes 1-9, using data obtained for sensitivity, specificity and limit of detection when compared to Real Time-PCR.

B169

Temperature change alters the plant root exudate metabolome and plant-microbe interactions in the rhizosphere

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Abstract

The make-up of the soil-root interface or rhizosphere is critical to plant health. The rhizosphere biota includes pathogens and parasites as well as symbiotic organisms which promote plant growth and can induce systemic resistance to some pathogens. One such organism is *Bacillus subtilis*, a symbiotic bacterium that produces protective biofilms around plant roots, releases toxins inhibiting fungal and nematode pathogens, and increases iron uptake. However, climate change is altering both the environments in which crops grow and the genes they express, potentially altering the symbiotic relationship between *B. subtilis* and its host plants. To sustainably protect crops it is important to understand the impact of climate change on the rhizosphere. The data presented here start to illustrate the impact of climate change on the plant root-*B. subtilis* relationship. We show that temperature change alters the composition of Tomato plant root exudate, which is critical to the recruitment of root symbionts such as *B. subtilis*. Analysis of the root exudate metabolome exposed a role for candidate compounds in recruiting or repulsing *B. subtilis* to/from the rhizosphere. Specifically, the root exudate compounds isopropyl stearate and 3,3-dimethylhexane both negatively impacted *B. subtilis* chemotaxis at biologically relevant concentrations. These compounds also significantly reduced the rate of *B. subtilis* biofilm formation with 3,3-dimethylhexane having the greatest impact. These findings start to unravel the potential impacts of climate change on the rhizosphere and will inform new approaches in crop breeding and gene editing efforts to optimise crop-microbe interactions for the promotion of sustainable plant health.

B172

Microbial remediation of microplastics within peat, soils, and coastal and riverine sediments

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Abstract

Plastics and microplastics (<5 mm) constitute a major pollutant in the biosphere. Whilst various studies have successfully isolated plastic-degrading microbes and characterised the rate and mechanism of plastic degradation, there is little known on the effects of microbial attachment and biofilm formation on the rate and efficiency of plastic degradation. This project uses a culture-based approach to isolate novel plastic-degrading microbes from the environment, based on the ecophysiology of microbes and their habitats, and quantifies plastic degradation in terms of gravimetric mass loss of four synthetic plastics: low-density polyethylene, polypropylene, poly(ethylene terephthalate), and polystyrene. Further, methods to enhance microbial attachment to the surface of the polymer (such as UV-C and non-thermal plasma pre-treatment of plastics) is also discussed.

Microbial habitats screened for plastic-degrading microbes include peat, lake sediment, reed-bed mud, and domestic compost. From these sampled habitats, a total of 43 bacterial species have been isolated and identified by 16S rRNA genes, including *Arthrobacter oryzae*, *Gordonia terrae,* and *Pseudomonas protegens*. Experiments to determine microbial degradation activity over a 28-day period indicate up to 20.8% mass loss of polystyrene by *P. protegens* and up to 22.73% mass loss of polypropylene by *G. terrae*. Further results give evidence of degradation of polypropylene and low-density polyethylene by other microbes.

Further work outlined in this poster includes determination of microbial cell surface hydrophobicity and biosurfactant production by plastic-degrading microbes. Additionally, whole genome sequencing of *G. terrae* facilitates a future genomic- and proteomics study to elucidate genes and enzymes involved in the depolymerisation of plastic.

B173

Potential use of purified sophorolipids subspecies in skincare and wound healing formulations

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Abstract

Surfactants are commonly used in skincare and wound healing formulations as emulsifying agents. However, most of these surfactants are of synthetic origin, and many have been reported to be a major cause of allergic reactions, skin irritations, and delay in wound healing. Hence, growth in consumers' demand for natural, sustainable, and biocompatible ingredients in skincare and wound healing formulations with better or equal performance to synthetic surfactants. In this regard, microbial sophorolipids (SLs) are a type of glycolipid biosurfactants mainly produced by *Starmerella bombicola* and exist as crude mixtures of lactonic (LSL) and acidic (ASL) forms. Glycolipids are well-known to possess bioactive properties such as antimicrobial, anticancer, and immunomodulatory effects. However, most *in-vivo* and *in-vitro* studies on these bioactive properties of sophorolipids were determined using their crude mixtures, resulting in significant inter-study variations, which are difficult to interpret. Therefore, to broaden the potential applications of SLs investigations, using their pure subspecies would be advantageous. In this study, we evaluated the cytotoxicity and wound healing effects of purified LSL (≈89.88%) and ASL (≈100%) on spontaneously transformed human keratinocytes (HaCaT) using XTT and *in-vitro* wound healing assays and compared them to synthetic surfactants (SLES). Cell viability assay revealed that up to 500 mg mL^{-1}, ASL were not toxic to HaCaT cells while in the *in-vitro* wound healing assay, both ASL and LSL significantly enhanced wound closure (99% and 71% closures respectively), making them potential candidates for use in skincare and wound healing formulations.

B174

Encapsulating bacteria to produce oxygenating biocoatings and artificial electroactive biofilms

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Abstract

The encapsulation of bacteria within colloidal polymers (biocoatings) enables opportunities for various interesting applications. Biocoatings are an excellent option when classical bioreactors are not suitable, given the excess biomass produced. They can be utilised for example in wastewater treatment processes, for sustainable energy production, and in the transport of functional microorganisms, where the polymer coating provides protection for the bacteria.

Different microorganisms were encapsulated in biocoatings to evaluate their viability. Functional biocoatings were prepared with *Escherichia coli* as a model organism, *Mycobacterium smegmatis* as a desiccation-resistant genetically tractable strain, the cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 to capture carbon, and electroactive bacterial communities able to produce electricity. To enhance diffusion, the porosity of the biocoatings was increased by adding halloysite nanotubes and adapting the hardness of the polymer particles. The biocoatings were film-formed by either full or partial desiccation.

The viability of the biocoatings was assessed by Confocal Laser Scanning Microscopy and either resazurin reduction assays or luminescence-based assays. Additionally, to assess the physiological function of the cyanobacteria, the production of oxygen was measured. Viable bacteria were found in higher proportions in the more porous biocoatings containing halloysite and in partially desiccated biocoatings, as the desiccation stress endured by the bacteria was reduced.

The optimisation of the polymer phase resulted in the formation of mechanically robust coatings and enhanced bacterial viability. Therefore, the formulation described could be used for a variety of bacterial species or communities, applicable to the fields of bioremediation and sustainable energy production.

B175

Evolution of environmental bacterial communities to further the degradation of waste plastic

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Abstract

Plastic waste is an increasing global issue with 12,000 million metric tonnes of plastic waste predicted to accumulate by 2050. An alternative to landfill and incineration is needed, with microbial biodegradation of plastic an increasing area of interest. Here, we not only aim to identify bacteria that can degrade plastic, but improve their capacity to do so for biotechnological application. Bacterial communities from polystyrene waste plastic were collected and displayed little or no plastic degradation, the polystyrene was then inoculated into non-carbonated minimal media for two months where the bacterial communities had only the waste polystyrene as a carbon source. This led to the evolution of the community from the selective pressure of needing to utilise polystyrene to survive. Comparison of the original and evolved bacterial communities found the evolved were quicker and more efficient at degrading polycaprolactone (PCL) agar, a polyester used to model plastic degradation, showing they have evolved into improved plastic degrading communities. The evolved communities use PCL as a sole carbon source resulting in weight reduction of the plastic. 16S sequencing found *Pseudomonas stutzeri* was dominant, with several strains sequenced, which through comparative genomics may identify enzymes involved in plastic degradation. Several *P. stutzeri* strains also reduce the weight of polystyrene pellets. This novel method to directly evolve environmental bacterial communities under the selective pressure of waste plastic as the sole carbon source has led to the development of strains that are more efficient at degrading plastic, providing a useful biotechnological tool in plastic waste disposal.

B176

Small RNA control of bacterial emissions of the greenhouse gas nitrous oxide

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Abstract

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential approximately 300-fold greater than carbon dioxide. Anthropogenic influences such as the use of nitrogen fertilisers have led to a significant increase in N_2O from microbial sources being emitted into the atmosphere. Subsequently, there is a pressing need for effective mitigation strategies targeting microbial N_2O emissions. As the genetic, physiological, and environmental factors regulating the microbial denitrification, a major pathway responsible for the biological production and consumption of N_2O are not fully understood, this represents a critical knowledge gap in the development of future mitigation strategies. RNA-seq analysis of the model soil denitrifier *Paracoccus denitrificans* has led to the discovery of 167 short regulatory small RNAs (sRNAs), many of which are differentially expressed under high and low N_2O emitting conditions. These include a number of sRNAs with potential FNR, NNR and NarR motifs in their promoter region suggesting they may be subject to regulation via these three major regulators of denitrification. Overexpression of a single sRNA, DenR, stalled denitrification through downregulation of nitrite reductase (NirS). Regulation via DenR is via a novel GntR type regulator of denitrification we have named NirR. Characterisation of further sRNAs and their regulatory networks in *P. denitrificans* will enable us to identify critical nodes in the denitrification regulatory cascade, as a route to novel mitigation strategies.

B177

Effects of biological and synthetic surfactants on antimicrobial resistance gene frequency in a streambed biofilm

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Abstract

Conventional wastewater treatment does not achieve complete removal of pollutants like surfactants from detergents and antimicrobial substances. Inland waters are frequently exposed to these chemicals from domestic, industrial, and agricultural sources. Surfactants affect the physical structure of streambed biofilms, and therefore also affect their interactions with other pollutants. Combined exposure with antimicrobials may then affect biofilm capacity to acquire and retain antimicrobial resistance genes (ARGs). Our study investigated the effects of a biological surfactant, rhamnolipid, and the commonly used synthetic surfactant sodium dodecyl sulphate (SDS), upon ARG retention within streambed biofilms. Biofilms were developed over a period of four weeks in contaminant exposure substrata experiments. Shotgun metagenomic sequencing was then performed on biofilm bacterial communities with an Oxford Nanopore Technologies MinION sequencer to identify ARGs and 16s rRNA. Although there were no differences in biofilm bacterial composition due to dominance by *Escherchia*, *Acidovorax, Janthinobacterium*, *Shigella*, *Flavobacterium,* and *Pseudomonas* spp. in all treatments and controls, the presence of ARGs was highest in surfactant treated systems. Rhamnolipid treatments contained the most types of ARGs (n=8) when compared to the control (n=4) or SDS (n=5) treatments. Only biofilm bacteria in SDS and rhamnolipid treatments had genes for tetracycline resistance genes. Rhamnolipid treated biofilms alone contained genes for resistance to elfamycin, isoniazid, and rifampin. Our results suggest that the contamination of aquatic ecosystems with surfactants such as rhamnolipids, and to a lesser extent SDS, may facilitate the retention of ARGs. Such pollution may thus contribute to proliferation of antimicrobial resistance in aquatic environments.

B178

Sustainable plastics: enrichment culture and metagenomic analysis of solvent rich waste system communities for the identification of candidate plastic platform chemical production hosts

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Abstract

The sustainable biosynthesis of platform chemicals is a promising route towards improving the sustainability of the plastics industry, but toxicity of these compounds is a factor hindering current efforts. Metagenomics of environments that are known to contain elevated levels of solvents can reveal solvent tolerant organisms as well as the tolerance mechanisms they wield. Here we took samples from an artist studio in which a variety of chemical compounds are used and washed away down the drains. We enriched the samples in R-2A media in cultures containing various concentrations of ethylbenzene, styrene, and a third compound of interest. Metagenomic sequencing was carried out for each sample using an Illumina Mini-Seq and used Kraken2 to perform taxonomic classification. Cultures containing our compound of interest had the most dramatic variation from the control sample almost entirely containing *Pseudomonas* species, in particular *P. putida.* Styrene cultures at concentrations of 5mM contained *Pseudomonas* species at 33% of the sample and when concentrations were at 10mM it decreased to around 2%, overtaken by *Acinetobacter* species at 37%. *Pseudomonas* species are well known for their tolerance to a range of solvents and antimicrobial agents, and this is well demonstrated with our compound of interest and will require further investigation to determine tolerance mechanisms. This is not reflected in our ethylbenzene and styrene groups in which *Acinetobacter* were dominant in each culture at higher concentrations. Using these organisms as a production strain or incorporating their tolerance mechanism into current strains may improve yields of tested compounds.

B179

Valorisation of Domestic Waste for the Production of Value-added Compounds

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Abstract

Domestic waste management and fuel availability are issues of vital importance for a sustainable future. Society's dependency on fossil fuels coupled with the dependency of single use substances, is pushing the natural and human environments to a critical point. Transitioning from a linear model of consumption to a sustainability focussed circular model, is an exigency if society is to meet the goals set out COP26. The building metabolism theme of the Hub for Biotechnology in the Built Environment is developing this circular economy by developing technologies for the conversion of inorganic waste products and feedstocks into sustainable and industrially relevant products within the building.

This project aims to help address these issues by combining the uses of metabolic engineering and fermentation processes to produce value-added products from waste feedstocks. The issues related to the upscaling and combining of biotechnological processes to have a complete cycle and move forward to its application.

The chemolithoautotroph *Cupriavidus necator* H16 will be engineered for the continuous use of H_2 and CO_2 to produce pre-cursor molecules, such as pyruvate, that will eventually be converted into biofuels with further engineering, CO_2 especially being one of the foremost waste gases currently. Once the strains are validated, they will be characterised in shake flasks and using gas fermentations to confirm the production of the desired products. The bioconversion pathways and fermentation conditions will be further optimised following further testing. This is an on-going investigation and preliminary results will be presented at the conference.

B181

Zinc and Antibiotic Resistance in Escherichia coli from livestock

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Abstract

Antimicrobial metal compounds may promote antibiotic resistance through co-selection of metal and antibiotic resistance genes carried on the same mobile genetic elements is currently a significant concern. Still, there is insufficient data on the co-presence of antibiotic resistance and metal resistance, epically zinc that was widely used in agriculture and husbandry. This study examined phenotypic resistance to 16 antibiotics and four metals (mercury, silver, copper, and zinc) of *E. coli* strains from pig sources in the United Kingdom. *E. coli* strains tested in this study were isolated from three different year periods, 2007-2010, 2017-2019, and 2020， almost covering the past 15 years. *E. coli* strains resistant to 4 mM zinc were isolated from zinc-treated pig groups. The resistance of strains from the control group and zinc-treated group indicated the promoting effect of zinc treatment on resistant strains. The results based on these strains would clearly show the changes of antimicrobial and metal resistance of strains sourced from different farms in different periods in the United Kingdom, would provide a wide range of data support, aid understanding the mechanism of co-selection between antibiotic resistance and metal resistance.

B183

Bottom-up design of synthetic algae-bacteria consortia for VOCs degradation and conversion into resource

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Abstract

With the advantages over single-species microbial systems recognised, microbial consortia are exploited in many industries. However, due to the lack of understanding about their complex biology, engineering microbial consortia with added functions remain difficult. Given the promising potential of microbial consortia, this study is motivated for bottom-up engineering of microbial systems that comprise phototrophic and chemotrophic microorganisms as a novel alternative for matching pollutant bioremediation with resource harvesting. In this study, two microalgae species *Deuterostichococcus epilithic*, *Coelastrella Terrestris* and 26 bacteria belonging to 8 genera that are resistant to specific volatile organic compounds (VOCs), including benzene, toluene, phenol, tetrahydrofuran and formaldehyde, were isolated from soil samples subjected to petrochemical pollution. These microbes were co-cultured in terms of algae-bacteria consortia that covered all possible combinations and were screened for further selection based on optical densities and chlorophyll fluorescence. Results show that both *D. epilithicus* and *C. terrestris* have resistance traits against VOCs mixtures. Two *Pseudomonas* and 5 *Rhodococcus* isolates were able to grow using VOCs as the sole carbon source while other VOCs-resistant bacteria showed no sign of VOCs utilisation. Consortia screening experiment reveal growth-promoting effects of different bacteria on algae *C.terrestris* which suggested efficient bacterial biodegrading of VOCs and VOCs-to-CO_2 exchange with microalgae as a mutualist interaction. Traversal consortia comparisons also profiled the correlation between algal biomass productions and consortia compositions. This study provides a benchmark for more universal microbes consortia engineering, gives useful references for both upscaled applications and insight on microbial interactions concerning synthetic algae-bacteria consortia.

B186

Acute Gastroenteritis in Children Below 5 Years in The Emergency Paediatrics Unit of a Tertiary Hospital, North-Central, Nigeria (2019 – 2021).

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Abstract

Acute gastroenteritis (AGE) is the highest cause of deaths in children below 5 years, globally with the most cases occurring in low-to-middle income countries (LMICs). AGE can be caused by helminths, protozoans, bacteria, and viruses, which account for most cases of AGE especially in LMICs; which, is associated with poor hygiene and nosocomial infections. Except for rotavirus, there are no approved vaccines against viral agents of AGE, neither are there antivirals against AGE. However, case management generally relies on symptomatic support and the administration of antimicrobial agents mostly misused in LMICs. Thus, surveillance remains a vital tool for proper management of AGE particularly in LMICs. A cross-sectional study was carried out to investigate 134 cases of AGE in children below 5 years that visited the emergency paediatrics unit of the University of Ilorin Teaching Hospital, Ilorin, Nigeria: from 2019 to 2021. Using real time RT-PCR, preliminary data shows an average annual prevalence of 95.43% (±0.75% S.D.) viral gastroenteritis that comprise monoinfections (~34%), co-infections (~30%) and super-infections (~32%) of up to 6 viral agents of AGE ($p<0.001$). Our data further suggests possible cases of nosocomial diarrhoea through the study period. Work is ongoing to apply next generation sequencing for characterisation of the microbial metagenomics associated with the cases of AGE.

B187

Bacteria in a five-species chronic wound-like biofilm have distinct growth dynamics with reduced metabolic activity.

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Abstract

AIMS:

The work was done to understand the inter-species interactions in a chronic wound biofilm model over a period of two weeks.

METHODS: The most commonly isolated species from chronic infected wounds were selected for understanding the mode of growth of multispecies biofilms occurring in chronic wounds, and that contribute to antimicrobial resistance (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis and Citrobacter freundii*). These species were grown in an agarose-collagen matrix supplemented with simulated wound fluid in a dynamic chronic wound biofilm model. The biofilms organisms were recovered at specific timepoints over two weeks, and community composition determined by total viable count (TVC) using selective agars. Staining and imaging of the biofilms by confocal and transmission electron microscopy (TEM) was undertaken to determine the growth pattern of species within the agarose-collagen matrix.

RESULTS: Biofilms grew in a steady state for two weeks with little difference in recovered bacterial numbers between species, but with reduced metabolic activity. Bacteria appeared to be growing in separate aggregates within the agarose-collagen matix as seen by confocal and TEM imaging.

CONCLUSIONS: Bacteria within this biofilm model appeared to grow in distinct niches that perhaps underpins the steady state of growth and consistent bacterial numbers over time. The reduced metabolic activity reflects the real-world situation and could be part of the mechanism by which biofilms in chronic wounds resist antimicrobial treatment.

B188

Human milk oligosaccharide DSLNT and gut microbiome in preterm infants predicts necrotising enterocolitis

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Abstract

Introduction: Necrotising enterocolitis (NEC) is a devastating intestinal disease primarily affecting preterm infants. The underlying mechanisms are poorly understood: mothers own breast milk (MOM) is protective, possibly relating to human milk oligosaccharides (HMOs), MOM microbiome, and infant gut microbiome interplay.

Methods: In a cohort of 49 NEC and 62 control infants MOM microbiome was determined through 16S rRNA gene sequencing, of which HMO profiling of MOM was performed for 33 NEC and 37 matched controls. Longitudinal stool metagenomic sequencing was also performed in a subset of 48 infants (14 NEC; n=644). Finally, bacterial species isolated from preterm stool were tested for their ability to grow on selected HMOs.

Results: Concentration of a single HMO, disialyllacto-N-tetraose (DSLNT), was significantly lower in MOM received by NEC infants compared to controls. On the contrary, no difference in MOM microbiome was observed. Metagenomic sequencing of infant stool before NEC onset showed significantly lower relative abundance of *Bifidobacterium longum* and higher relative abundance of *Enterobacter cloacae* in infants with NEC. Moreover, infants receiving low MOM DSLNT were associated with reduced transition into preterm gut community types dominated by *Bifidobacterium* spp. Numerous bifidobacteria isolated from preterm infants showed growth on selected HMOs, although no isolate tested could metabolise DSLNT.

Conclusion: These results demonstrate the importance of HMOs and gut microbiome in preterm infant health and disease, and that DSLNT may improve outcomes by potentially microbiome-independent mechanisms. The findings offer potential targets for biomarker development, disease risk stratification, and novel avenues for supplements that may prevent life-threatening disease.

B189

Disrupting social messaging in polymicrobial communities

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Abstract

Quorum sensing (QS) via small, diffusible signalling molecules known as autoinducers allows bacteria to collectively monitor their surroundings and respond accordingly. QS controls many bacterial processes, including virulence factors such as biofilm formation, swarming motility and extracellular protein/molecule production. These autoinducers can also facilitate intra-species, inter-species, and inter-kingdom communication as microbes are often co-located within diverse polymicrobial communities**.** Why and how microbial 'bad actors' emerge from these diverse communities to cause dysbiosis and infection remains to be established. However, elucidation of potential links with QS autoinducers may provide new opportunities for anti-infective development to combat the challenge of antimicrobial resistance.

New anti-infective and anti-virulence strategies have the potential to target key community-coordinated behaviours that underpin the harm caused by microbial pathogens, such as biofilm formation, secretion, and toxin production, without causing dysbiosis of the microbial community. Rather than seeking to disrupt the native QS signalling systems of bacteria, a novel approach is to use these competitive signalling systems as a framework for control of co-colonising pathogens.

A series of synthetic analogues of known QS signals were studied for their ability to interfere with virulence behaviour of ESKAPE pathogens including *Pseudomonas aeruginosa* and *Staphylococcus aureus* within mixed microbial populations of increasing complexity**.** Different synthetic modifications of the same QS backbone were found to elicit a distinct response at the species level, further evidence that tailored design of behavioural modulators is possible.

B190

Cell Invasion by the key oral bacterium *Fusobacterium nucleatum*

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Abstract

Background

Fusobacterium nucleatum (FN) is an important commensal anaerobe, playing a bridging role in the formation of polymicrobial, oral biofilms. Whilst especially abundant in oral diseases such as periodontitis (gum disease), it has also been linked to a plethora of extra-oral diseases such as colorectal cancer (CRC). Its association with such a range of diseases is thought to be a result of its invasive nature, using in particular the Fusobacterial Adhesin A (FadA). Even though FN invasion has been studied to some extent, there is a gap in the literature regarding the five FN subspecies. These are characteristically distinct, yet often studies focus on only one (often-undefined) subspecies. Here, we aim to characterise oral epithelial cell (OEC) invasion by different FN ssp.

Methods

Three subspecies were chosen due to their invasion and mechanistic role in CRC (ssp *nucleatum*, ssp *animalis*, and ssp *polymorphum*). These were studied individually and in combination with commensal (*Streptococcus sanguinis*) and pathogenic (*Porphyromonas gingivalis*) oral bacteria. H400 squamous cell carcinoma cells were used as a model cell-line for OEC. Data was gathered using apoptosis assays, invasion and adhesion assays, and scanning electron microscopy.

Results

Differences in adhesion, invasion, and induced apoptosis by FN ssp will be presented. These results will be compared to those from a ΔFadA mutant, as well as those from polyinvasion with *S. sanguinis* and *P. gingivalis*.

Conclusions

This study provides new insight into the mono/polyinvasion of different FN ssp into OEC. It aims to dissect the mechanism, examining the role of FadA in invasion.

B191

Alteration of the cutaneous microbiome in Epidermolysis bullosa and its potential role in wound healing.

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Abstract

The skin is the largest human organ. Interfacing with the surrounding environment, it acts as a physical barrier protecting internal organs from infection and trauma, including the damaging effects of sunlight. Its microbiome, pH, structural and thermoregulatory properties, and resident immune cell population contribute to making the skin an active organ of the immune system. Alterations in skin barrier components may result in pathogenic conditions such as skin infection, inflammation, allergic reactions, cutaneous cancers, or genetic diseases such as Epidermolysis bullosa (EB). EB is the prototypic and heterogeneous group of bullous disorders characterised by detachment of the epithelium following minimal mechanical trauma. EB is classically grouped into four major types according to the level of skin cleavage: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler EB (KEB). A heterogeneous phenotypic spectrum exists with: persistent blistering, inflammation, delayed re-epithelisation, abnormal wound healing, and infection. It can cause disability and death. Currently, there is no cure.

Understanding the skin microbiome and its relationship to the inflammatory-immune response are fundamental prerequisites for developing novel therapies. We employed whole-genome sequencing to investigate and characterise the microbiome from skin swabs. Metagenomic sequence data were processed through the MG-RAST (Metagenomics-Rapid Annotations using Subsystems Technology) pipeline. We identified shifts from a health- to disease-associated skin microbiome in three groups of EB (EBS, JEB, and DEB) which will be presented. This shift may be involved with atypical wound healing and understanding it further may present opportunities for improved intervention, leading to improved quality of life.

B192

Inter-species interactions alter antimicrobial susceptibility in polymicrobial cultures.

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Abstract

Pseudomonas aeruginosa (PA) is an opportunistic human pathogen. The WHO recently designated PA as a "critical priority pathogen" against which new antibiotic interventions are urgently needed. The biology of PA has been extensively studied in mono-species cultures, yet during many infection scenarios, the organism frequently finds itself sharing the niche with other microbial species. This is especially so in airways of people with cystic fibrosis (CF), where the organism co-habits with a veritable "zoo" of other microbes, including Gram-negative, Gram-positive and fungal species. The problem is that such microbial consortia are difficult to stably maintain *in vitro*. However, recent advances made in our laboratory have enabled us to co-culture PA with a variety of other microbes. Using this *in vitro* continuous-flow system, we can now begin exploring how inter-species interactions alter the biology of PA.

We have uncovered strong evidence for extensive inter-species interactions between PA and its neighbours. For instance, co-culturing PA with other microbes dramatically enhances its resistance to antibiotics; whereas the growth of PA in mono-species cultures is strongly affected by even one dose of an anti-pseudomonal antibiotic (colistin), the presence of two other common CF-associated species, *Staphylococcus aureus* and *Candida albicans*, confers a much higher degree of protection. The question is, what happens if another antibiotic is applied or additional CF-associated species are introduced into the culture? How do these affect antibiotic susceptibility, and what are the molecular mechanisms involved? These questions will be explored in more detail in my presentation.

B193

Sialidase of *Tannerella forsythia* Upregulates Pro-inflammatory cytokines and is inhibited by di-fluoro sialic acid 2e3aDFNeu5Ac9N3

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Abstract

Periodontitis is a chronic inflammatory disease affecting the tissues surrounding and supporting the teeth. Porphyromonas gingivalis and Tannerella forsythia are important pathobionts in periodontitis shown to modulate host immune system. Here we tested the hypothesis that inhibition of the sialidases of these organisms might modulate cytokine production of epithelial cells with which these bacteria interact during infection. In this work we used a chemoenzymatically synthesized di-fluoro sialic acid analog 2e3aDFNeu5Ac9N3 that is known to have inhibitory activities against other bacterial sialidases and the recombinant human cytosolic sialidase hNeu2 (Li et al., 2019). Inhibition studies using whole cells and the sialidases of T. forsythia (TF) and P. gingivalis (PG) showed 2e3aDFNeu5Ac9N3 to have potent activity. Using purified NanH it was established that it's mechanism of action was as a competitive inhibitor. Furthermore, it was not toxic to oral epithelial cells at the concentrations at which it inhibits these enzymes (1µM) and at which it inhibits invasion of these cells by TF/PG (1 and 4µM).

Additionally, innate immune responses of H357 oral epithelial cells in the presence or absence of 2e3aDFNeu5Ac9N3 and TF(ATCC43037)/PG(0381) or using a strain lacking sialidase were assessed using flow cytometry. This revealed that sialidase activity appears to modulate levels of several pro-inflammatory cytokines; namely interleukin-6 (IL-6), IL-8 and IL-1β in the cell supernatants.

Summarily, the sialidase of T. forsythia upregulated cytokine secretion in H357 cells which was abrogated significantly by 2e3aDFNeu5Ac9N3 with minimal cytotoxic effects on the oral epithelial cells. This compound may have potential for future development.

Sialidases, Tannerella forsythia, Porphyromonas gingivalis, Cytokines, 2e3aDFNeu5Ac9N3

B194

Gut microbial stability is associated with greater endurance performance in athletes undertaking dietary periodisation.

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Abstract

Objective

Environmental stressors can promote temperate phages to switch from lysogenic to lytic life-cycles. High-level athletes undergo dietary modulation during training which causes substantial gut stress. This study explores the impact of acute high protein or carbohydrate diets on measured endurance performance and associated gut microbial community changes.

Design

In a cohort of well-matched, highly trained endurance runners, we measured performance outcomes, as well as gut bacterial, free viral particles (FVP) and inducible lysogenic bacteriophage (IV) communities in a double-blind, repeated-measures design RCT to explore the impact of dietary intervention with either high protein or carbohydrate content,

Results

High dietary carbohydrate improved performance during time-trial by +6.5% ($p < 0.03$) and was associated with expansion of *Ruminococcus* and *Collinsella* bacterial spp. Participants whose performance increased most had greater proportions of functional genes associated with energy utilization.

High dietary protein led to a 23.3% performance reduction ($p = 0.001$), significantly reduced diversity (IV: $p = 0.04$) and altered composition (IV and FVP: $p = 0.02$) of the gut phageome. Participants who experienced greatest shifts in community composition also performed worst during dietary modification.

Conclusion

Gut microbial stability during acute dietary periodisation was associated with greater athletic performance in this highly-trained, well-matched cohort. Athletes and those supporting them should be mindful of the potential consequences of dietary manipulation on gut flora and implications for performance and periodise appropriately. Bacteriophage populations offer a better marker of microbial stability than their bacterial hosts.

B195

Polymicrobial *In vitro* Bladder Model Development for Studying Catheter Associated Urinary Tract Infections

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Abstract

Urinary catheters are one of the most commonly deployed medical devices, used extensively for long term bladder management in hospitals, residential care homes and the community. Catheter associated urinary tract infections (CAUTIs) are highly prevalent hospital acquired infections, placing significant burden on healthcare institutions. The majority of CAUTIs are mixed bacterial communities of up to 5 species but *in vitro* studies in the literature often focus on single species studies. This study describes the development of a polymicrobial *in vitro* bladder model, which is representative of an infected, catheterized urinary tract. This model was originally described by Stickler *et al*., (1999) and has since been applied to study commonly isolated urinary tract pathogens. This model replicates a closed drainage system as used in clinical practice, and simulates physiological parameters (*e.g.,* temperature and flow rate). This allows the establishment of CAUTIs and formation of biofilm communities under conditions which more closely represent *in vivo* conditions. Here, this model has been adapted for use with polymicrobial communities, establishing a reproduceable mixed species community of the catheterized urinary tract. In this study culture-based, differential, and selective methods are utilised for bacterial enumeration as well as phenotypic and genomic analysis allowing evaluation of the polymicrobial community dynamics. The development of protocols to model polymicrobial CAUTI will allow us to address important questions regarding the evolution of virulence, biofilm formation, and antimicrobial resistance in these important microbial communities.

B196

Bacteriocin-mediated conflict by *Staphylococcus aureus* in the human nasal cavity.

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Abstract

Staphylococcus aureus asymptomatically colonises the nasal cavity of one in three adults. Interestingly, while some hosts are colonised by one *S. aureus* strain over time, others are co-colonised by multiple strains, each with varying degrees of colonisation success. Competition between strains, and with other species, may be important in determining colonisation success in these natural populations. One way bacteria can mediate such competition is by producing antimicrobial toxins called 'bacteriocins' that kill unrelated strains and species. However, we are yet to understand the extent to which bacteriocins mediate competition and drive long-term ecological and evolutionary success in natural populations of *S. aureus*. Here, we tested this by screening 501 *S. aureus* nasal isolates from 40 participants sampled over 90-months for their ability to produce bacteriocins against other *S. aureus* nasal strains and commensal species that co-inhabit the nasal cavity**.** We found that ~20% of S. aureus strains could produce bacteriocins and that they play an important role in determining *S. aureus* colonisation success: strains from single-strain participants, which are relatively successful colonisers, are more likely to produce bacteriocins than strains from co-colonised participants. *S. aureus* bacteriocins were also significantly more effective against other co-inhabiting species compared to other *S. aureus* strains, demonstrating the relatively high prevalence of antagonistic interspecific competition in the human nasal cavity.

B197

Characterisation of a novel Type VI secretion system associated lipoprotein from *Serratia marcescens*

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Abstract

The bacterial Type VI secretion system (T6SS) is a dynamic macromolecular structure that promotes inter- and intra-species competition through the delivery of toxic proteins into neighbouring cells. The T6SS from *Serratia marcescens* contains thirteen well characterised "core" proteins (TssA-M) that are required for T6SS-mediated toxin delivery. Using an established assay for interbacterial competition, we have identified a novel accessory component required for optimal T6SS firing which we have named Type VI accessory lipoprotein 1 (Tal-1) and characterised the association between Tal-1 and the core T6SS component TssJ. Deletion of *tal1* reduced the T6SS-dependent antibacterial activity of the *S. marcescens* model strain Db10 by approximately 100-fold. Mutants containing a deletion in *tssJ*, previously considered essential for T6SS firing, retained a modest T6SS activity that was abolished upon deletion of *tal1*. Tal-1 did not interact with the T6SS membrane complex proteins TssL and TssM suggesting that its mechanism of action is distinct from that of TssJ. Homologues of *tal1* have been identified in several other genera of Gram-negative bacteria, suggesting that the accessory function of this protein is not restricted to *S. marcescens*. Together these data suggest that a second, TssJ independent mechanism for T6SS firing may exist that is dependent upon the activity of Tal-1 and its homologues.

B198

How *Pseudomonas aeruginosa* uses Type VI Secretion system and range of toxins to gain advantage in polymicrobial communities

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen and its biofilm lifestyle is associated with chronic infections. To establish and persist within context of dense polymicrobial communities *P. aeruginosa* utilises Type VI Secretion system (T6SS) for direct competition. T6SS is a macromolecular weapon used to inject toxic effectors directly into adjacent prey cells. *P. aeruginosa* possesses 3 distinct T6SSs and a set of more than 20 toxic effectors. T6SS toxins possess a diverse set of functions including degradation of nucleic acids, disruption of prey metabolism or inducing prey lysis by disrupting membrane and cell wall integrity.

We generated a collection of mutants sensitive to individual toxins and lacking T6SS machinery aiming to investigate how *P. aeruginosa* gains a competitive edge in polymicrobial communities. By imaging whole mixed bacterial macrocolonies, it is possible to investigate how community structure is affected by the action of individual T6SS toxins. Additionally, disruption of Gac-Rsm cascade, responsible for the transition between planktonic and biofilm lifestyles and modulation of T6SS activity, results in both graduated elevation in T6SS expression and a corresponding increase in T6SS dependent killing. Experimental work is supported by use of agent-based theoretical simulations that allow to connect how changes in specific T6SS firing behaviours at single-cell scale lead to population-level competitive advantages. Altogether this will allow to understand how highly localised contact-based interactions between individual bacteria shape the structure of whole populations and how *P. aeruginosa* uses its substantial T6SS arsenal to successfully eliminate prey.

B199

The cystic fibrosis airway microbiome: quantifying Pseudomonas aeruginosa interactions with other microbes.

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Abstract

Cystic Fibrosis (CF) is a genetic disease that is associated with exuberant production of secreted airway mucus. This mucus acts as an excellent substratum for a variety of microbes, mostly infamously, Pseudomonas aeruginosa (PA). PA infections often last for decades, and are associated with a progressive decline in microbiome diversity, and a decline in patient prognosis. However, we still do not understand why PA outcompetes the other microbiota. The aim of this project is to understand better how PA interacts with the other microbiota; are the interactions competitive, synergistic or neutral? To address this, we have developed a Bayesian model based around the Lotka-Volterra Competition relationship. The model allows us to extract pairwise "species interaction coefficients" in multi-species ecosystems. We are currently applying this model to understand inter-species interactions in an in vitro co-cultivation model containing three CF-associated pathogens; PA, Staphylococcus aureus (SA) and Candida albicans (CA). Our data indicate that PA competes with SA more intensely in steady-state co-cultures compared with pre-steady-state co-cultures. By contrast, PA always competes with CA irrespective of co-culture status. Similarly, SA always competes with CA, although the latter eventually gains the upper hand in the longer-term to establish low, but stable titres in the steady-state. Currently, we are also applying the model to investigate inter-species interactions in clinical samples using longitudinal data from the CF registry. A better understanding of how species interact in the CF airways may eventually lead to improved therapeutic interventions aimed at maintaining microbial diversity in the CF airways.

B203

Recreating a unique early modern beer

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Abstract

FOODCULT is an ERC project exploring interconnections between food, diet and culture in medieval Ireland (c1550 – c1650). It takes an interdisciplinary approach that merges micro-historical analytical techniques with experimental archaeology to examine what was eaten, where, why and by whom. It was already known that beer was an important source of nutrition and the archival records in Dublin Castle provide extensive details of ingredients and recipes of the time. This facilitated an attempt to recreate an Irish medieval beer to examine its nutritional composition to help ascertain the overall contribution of beer to diet. As well as barley, Irish beer included oats and hops. To recreate this beer as closely as possible, heritage cereal varieties and hops were sourced from around the UK and a beer was made following the original recipe and ingredients. For this, a working Tudor brewery that used traditional equipment and processes was built at the Weald & Downland Living Museum in Sussex. Selecting the most appropriate yeast strain was a challenge as that era precedes pure cultures by several hundred years. We took advantage of data from the NCYC yeast culture collection to source two strains that are genetically close to the root of the tree of ale yeasts. The candidate strains were screened for a number of traits and one chosen to represent the ancestral strain. The fermentation process was monitored and the beer analysed for nutritional parameters. We will present an integrated update of the science and the history of this ongoing project.

B204

Seasonal trends amongst genotypes of Listeria monocytogenes from clinical cases of listeriosis, food and the environment

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Abstract

Since 2015, the UK Health Security Agency has applied whole genome sequencing for the characterisation of *Listeria monocytogenes* from severe clinical cases, as well from food and food production environments. Thus, UKHSA holds one of the largest genomic surveillance databases containing WGS sequences are associated meta-data.

L. monocytogenes is a food-borne pathogen causing listeriosis. Transmission is most common from contaminated foods and typically affects patients over 60 years of age, the immunocompromised as well as the pregnant women, their unborn and newly delivered babies [1]. In these high-risk groups listeriosis causes a severe, life-threatening, systemic infection. Thus, *L. monocytogenes* is the leading cause of death attributed to food-borne disease in England. Using the UKHSA database, this work aims to identify the genomic markers associated with virulence and persistence in food production environments, and aims to assist in disease risk assessment and management.

A descriptive analysis of the UKHSA's listeria database has been performed, investigating *L. monocytogenes* distribution in the UK.

The results show that *L. monocytogenes* belonging to lineage I is more commonly associated with clinical disease, whilst cultures within lineage II are more frequently isolated from food and food production environments. The distribution of lineages and clonal complexes showed increases in severe clinical cases during the summer and autumn, unlike isolates from food and the environmental isolates which showing peaks in the autumn and winter. Further investigation is ongoing to explain the seasonality trends.

These data provide an initial analysis exploiting the genomic resources held by UKHSA on L. monocytogenes.

B206

Faecal metabolome and microbiota evaluation of dogs with Canine Cutaneous and Renal Glomerular Vasculopathy using 1H NMR and 16S rRNA gene profiling

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Abstract

Canine Cutaneous and Renal Glomerular Vasculopathy (CRGV) is an idiopathic, usually fatal disease characterised by skin lesions and, in many cases, acute kidney injury. The aetiology of CRGV is currently unknown, hindering the development of effective interventions. In this study, the faecal metabolome and microbiota composition were examined for healthy (n=96) and CRGV-affected dogs (n=104) using 1H Nuclear Magnetic Resonance (NMR) spectroscopy and 16S rRNA sequencing, respectively. Microbiota profiling was conducted following DNA extraction from faecal samples; the V4-V5 regions of the 16S rRNA gene were amplified and subsequent amplicons sequenced (MiSeq). Metabolomic profiling was carried out on faecal water using a 700 MHz Bruker NMR spectrometer and the spectral profiles were interrogated using multivariate statistical analysis tools in MatLab. To classify taxonomic profiles, sequences were processed generating Amplicon Sequence Variants (ASVs) via QIIME2 using DADA2 quality control, providing a higher resolution of the microbiota composition in comparison to Operational Taxonomic Units (OTUs). Data analysis revealed Alpha diversity (measured as Shannon diversity) was not significantly different between healthy and CRGV-affected dogs ($p>0.05$). The community profile of CRGV-affected dogs revealed differences in the structure when compared to healthy dogs, with increases in the bacterial families Enterococcaceae and Enterobacteriaceae in the diseased dogs. This study also identified a number of metabolites linked to bacterial metabolism that differed between the CRGV-affected and healthy dogs. The findings may offer a potential explanation for the pathology seen in CRGV. This is the first study to characterise the faecal microbiota and metabolome of CRGV-affected dogs.

B207

Flu-GLUE: A data-centric bioinformatic resource for analysing influenza virus sequence variation

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Abstract

Analysis of influenza virus sequence data is a fundamental part of assessing diversity within influenza virus strains and informs vaccine strain selection, increases knowledge of host-specific adaptations and understanding of viral protein variations and interactions. However, the need to understand diversity has led to exponentially increasing deposition of sequences within databases which brings challenges with sequence data handling. Further, there is currently no efficient way to navigate sequences and assess variation at any specified site in an influenza virus' genome/proteome. To address these issues, we have used the GLUE (Genes Linked by Underlying Evolution) framework to develop Flu-GLUE. GLUE provides a flexible, bioinformatic environment to build resources dedicated to organising viral sequence data and has been used previously for other viruses such as HCV. Flu-GLUE is a computational resource that effectively manages the GenBank records of sequence data for all four genera of influenza virus and provides simple, efficient commands for downstream data processing and analysis. We demonstrate the utility of Flu-GLUE, by describing sequence variation at well-characterised, host-specific residues in viral proteins, as well exploring variation in phosphorylation sites. Flu-GLUE readily returns multiple sequence alignments (MSAs), phylogenetic trees, tabular data and sequence motifs describing how the specified sites vary across all publicly-available influenza virus sequences. Both experienced bioinformaticians and users with no formal bioinformatic training can use Flu-GLUE to, readily and reproducibly, analyse increasingly challenging volumes of influenza genome sequence data, providing versatile and streamlined analysis for defined, user-specified questions.

B208

Longitudinal studies to risk assess microbiome perturbations induced by the application of cosmetics which target the microbiome

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Abstract

The residents of a healthy skin or oral microbiome help prevent invasion by pathogenic species and demonstrate resilience despite frequent perturbations from cosmetic and hygiene practices (McBain et al., 2019). To understand the potential effect of cosmetic technologies which target the microbiome (e.g. antimicrobials, pre/probiotics, phage-therapies) on microbiome balance, we need to study the effect of these perturbations.

We propose a microbiome safety framework to risk assess the likelihood of dysbiosis after application of such cosmetics, based on the notions of history of safe use, resilience to short term perturbations and ultimately, when scientific knowledge permits, maintenance of microbiome functions that must be protected to enable a healthy host state. When no history of safe use data are available, longitudinal human studies can be used to characterise the time-related notions of resistance, resilience and dysbiosis.

Several studies have demonstrated resilience to antimicrobial treatment (SanMiguel et al., 2018, Saville et al., 2022), and that the recovery of microbiome diversity and abundance happened between 6 and 24 h after treatment. If, after a perturbation, the microbiome composition does not return to the baseline, we propose that more research is required using Next Generation Sequencing and -omics data, to characterise microbiome functions that need to be protected to maintain a physiologically healthy status in the host, by measuring temporal trends.

It is the first time an approach for assessing microbiome-related consumer safety is being presented and some directions for future research to understand the dysbiosis endpoints are proposed (Metris et al., 2021).

B209

Exploring chicken caeca microbiomics for novel antimicrobial discovery

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Abstract

There is critical need for new therapeutics for the treatment of clinically relevant drug resistant pathogens, especially those in the ESKAPES group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species* and *Stenotrophomonas maltophila*). Gut microbiomes are an abundant resource for novel bioactive discovery including antimicrobial peptides (AMPs). Here, we present the chicken caeca microbiome as a source for novel antimicrobials specifically against Gram negative pathogens.

A total of 6 chicken caeca transcriptomic datasets were prospected for potentially novel antimicrobial peptides using in silico and combinatorial approaches. We identified ~110 AMPs which, were screened for antimicrobial activity against a plethora of clinically relevant pathogens. The therapeutic potential of four of the most promising chicken caeca derived AMP candidates, namely C-9, C-55, C-77 and C-103 against clinical strains of a most troublesome pathogen *Acinetobacter baumannii* was investigated. All four AMPs inhibited growth of *A. baumannii* strains with MIC concentrations between 4-16 µg/ml. They induced rapid loss of cell viability (> 9 log CFU/ml reduction) after only 5 minutes for C-9 and C-103 and > 4 log CFU/ml reduction after 30 minutes of treatment (C-55 and C-77 respectively). This killing was characterized by rapid permeabilization of the cytoplasmic membrane by AMPs and loss of membrane integrity. A high therapeutic index with little cytotoxicity against mammalian cells also suggests their safety in intravenous use and their potential as drug leads/templates for the treatment of *A. baumannii* and other bacterial infections.

B211

A novel therapy for urinary tract infections

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Abstract

Serious bacterial infections represent an unprecedented worldwide threat, due partially to the emergence of antibiotic-resistant bacteria for which we have limited therapies. A key aspect of the antibiotic resistance problem is the ability of bacteria to form biofilms, which provide protection from both antibiotics and the host immune system. Biofilms are implicated in chronic infections such as tuberculosis, cystic fibrosis-associated lung infections and urinary tract infections (UTIs). UTIs are among the most frequent bacterial infections affecting 150 million people per year worldwide and 75% of infections are due to uropathogenic *E. coli*. A major problem with UTIs is recurrence of infection associated with the ability of *E. coli* to form biofilms on the bladder lining or on indwelling catheters. Recent work in our laboratory and elsewhere, has demonstrated a role for the enzyme tryptophanase in the formation of *E. coli* biofilms. Therefore, inhibition of tryptophanase offers an attractive and plausible route to reduce pathogenicity and increase antibiotic effectiveness for infections by uropathogenic *E. coli*. Combining high-throughput assays with *in silico* screening, in collaboration with Cresset (https://www.cresset-group.com/), a UK-based computational chemistry provider with specific expertise in screening *in silico* for modulators of enzyme activity, we have identified a set of novel tryptophanase inhibitors and, in a proof of concept, have confirmed their ability to inhibit biofilm formation in clinical strains of uropathogenic *E. coli*. Our project aims to generate effective biofilm inhibitors to be used ultimately in the treatment and management of recurrent UTIs.

B212

Antimicrobial efficacy of ruthenium compounds: developing novel antimicrobials to combat resistance

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Abstract

The World Health Organisation has declared antimicrobial resistance (AMR) as one of the top 10 global health threats and estimates 10 million deaths annually attributed to AMR by 2050. Mono and di nuclear ruthenium complexes offer a novel solution to relieve the burden posed by AMR infections showing excellent antimicrobial activity against multi-drug resistant pathogens including *Escherichia coli* EC958.

Minimum inhibitory/bactericidal concentrations assays used to determine the antimicrobial activity of ruthenium compounds. Time-kill assays and various imaging techniques including structured illumination microscopy (SIM), TEM and STEM performed to probe the effect on actively growing EC958.

Significant activity is shown against clinically isolated multidrug-resistant pathogens with concentrations comparable to standard antibiotics (2.9mg/L [1.6µM] against EC958). Both compounds are also effective across a broad pH range (4-9). Time-kill assays demonstrate a lag-phase (~30-60min) after the introduction of compound where no change in turbidity is seen with structured illumination microscopy demonstrating di nuclear ruthenium accumulates within the cell membrane 20 min post-exposure before concentrating inside the cell.

Ruthenium complexes show excellent potential as novel antimicrobials with a potent broad range of antimicrobial activity across bacterial species and pH ranges. This demonstrates the effectiveness of the compounds across a wide range of infections where environmental conditions may change throughout the infection time-course. We later aim to establish a greater understanding of the mechanism of action through a full transcriptomic study.

B213

Genomic characterisation of *Escherichia coli* causing early and late onset neonatal sepsis in Irish maternity hospitals.

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Abstract

Escherichia coli (*E. coli*) has emerged as the leading cause of gram-negative sepsis in preterm neonates, and contributes to significant morbidity and mortality on a global scale. Whilst several large sequencing studies have provided detailed analysis of the population structure of these isolates in adults, equivalent data is lacking in the paediatric population. As a result, the phenotypic and genotypic profile of *E. coli* associated neonatal sepsis is not well characterised in Ireland. By combining epidemiological, phenotypic and genotypic data our study aims to enhance our understanding of *E. coli* associated early-onset and late-onset sepsis.

E. coli strains isolated from confirmed cases of early-onset sepsis and late-onset sepsis were collected by participating maternity hospitals. A total of 150 cases and controls were subjected to Illumina sequencing and were linked to routinely collected electronic healthcare records and mandatory surveillance reports. Bioinformatic analysis facilitated multi locus sequencing typing (MLST), the identification of virulence factor (VF) genes and antibiotic resistance genes. Contrasting the distribution of these genetic determinants with clinical factors will expand our knowledge of the molecular epidemiology of *E. coli* associated early-onset and late-onset sepsis in Ireland.

B214

The impact of the metabolic landscape of the human gastrointestinal tract on pathogenesis of *Streptococcus gallolyticus*

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Abstract

The pathophysiology of gut bacterium *Streptococcus gallolyticus* subsp. *gallolyticus* (*Sgg*) remains largely unknown. It is an opportunistic pathogen that has been shown to colonise colorectal cancer tissues and, following colonisation, translocation occurs through the intestinal barrier into the bloodstream. *Sgg* were previously believed to be non-motile, however, this study shows that *Sgg* are motile bacteria, that may utilise type IV pili for twitching motility. This novel phenotype is abolished in *Sgg* in response to growth in different carbon sources, but not bile or amino acids. We show that Pil1 of *Sgg,* of which is responsible for collagen adherence, among other functions, is not affected by growth in different sugars. We have confirmed the presence of all essential genes present in the *com* operon that we propose is required for the observed motility. We show that *Sgg* adhere to and invade colonic cancer cells, with a reduction in invasion when glucose is present. Based on the results from this study, we hypothesise that *Sgg* regulates motility in response to the metabolic landscape of the human gut to maximise nutrient acquisition, enhance colonisation and ultimately exploit cancer cells resulting in systemic dissemination.

B215

Isolation and characterisation of *Proteus* bacteriophages for the development of therapeutic phage cocktails

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Abstract

Proteus mirabilis is a common opportunistic human pathogen mainly responsible for UTIs, including catheter-associated ones. It is characterised by a number of unusual traits, including its swarming activity and the ability to form crystalline biofilms, causing encrustation and eventual blockage of urinary catheters colonised by *Proteus* spp. This could lead to the ascension of bacteria up the urinary tract and result in chronic, recurring infections. *P. mirabilis* is naturally resistant to numerous antibiotics, including colistin, and drug-resistant isolates are increasingly being reported. If this trend continues, *P. mirabilis* threatens to become a dangerous multi-drug resistant pathogen, which would cause significant treatment difficulties. Bacteriophage therapy is considered one of the most attractive alternatives to the use of antimicrobial agents for treatment of multi-drug resistant bacterial infections. In this project, we aimed to identify and characterise new bacteriophages that could be directly used for biocontrol of catheter-associated UTIs caused by *Proteus* spp. in the form of phage cocktails and also serve as a potential source of endolysins and depolymerases. The isolation and purification of phages were carried out by spot test and double layer plaque assay from environmental samples collected from different locations in Northern Ireland. The phages isolated so far have been extensively characterised and form a foundation of a phage bank against a range of *Proteus* spp. Further investigation of the collected bacteriophages and their ability to prevent the formation and/or eliminate *Proteus* biofilms will allow us to identify the most promising candidates for the development of therapeutic formulations.

B216

A porcine skin infection model to study novel treatments for dermatophytosis

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Abstract

Dermatophytes are fungi that cause infections of skin, nails, and hair, with the most common causative agent being *Trichophyton rubrum*. The infections have a high prevalence of about 20-25% of the world's population, and these are often recurrent due to either relapse or reinfection. There is also an increased incidence of failure of antifungal treatments, and novel prevention and treatment strategies are highly desirable. We have developed an infection model using explanted porcine skin, which is a good mimic of the human skin because of the similarities of structure and thickness. In the porcine skin model, fungal growth depends on the presence of skin with the concomitant production of keratinases, which are important virulence factors in dermatophytes. We have employed this model to study the early steps in the infection process, such as adherence of fungal spores to the skin. However, the porcine skin model lacks an immune system, and later stages result in luxurious fungal growth that is not seen *in vivo*. The model is very useful to study treatments of dermatophytosis, and we demonstrate this with clinically used antifungal agents as well as novel treatment strategies. The latter includes, for instance, antifungal polymers, which have multiple modes of antifungal action, including interaction with the cell envelope and chelation of essential metals.

B217

Novel Anti-Mycobacterial Compounds Uncovered Through Drug Repurposing

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Abstract

Drug repurposing is gaining popularity as a scientific endeavour to discover new anti-mycobacterial therapies and treatment options for tuberculosis. However, the majority of these research efforts are primarily focused upon repurposing existing antibacterial drugs to combat infection, rather than approved drugs used in other clinical indications.

To this end, a high-throughput chemical screen of currently approved drugs (the Prestwick library), identified several molecules with potent anti-mycobacterial activity, against *M. smegmatis* and *M. bovis* BCG. In this study, we have conducted a detailed mode of action analysis on one of these hits. This compound displays specific anti-mycobacterial activity, with no inhibitory activity towards a majority of the ESKAPE pathogens. Thus far, we have been unsuccessful in attempts to generate drug-resistant mutants for follow up genomic sequencing, revealing that this drug exhibits an undetectable frequency of resistance. Further mode of action determination is currently ongoing, with stark changes to the *Mycobacterial* cell envelope occurring within six hours of drug treatment. The target is expected to be *Mycobacterial* or Gram-positive specific rather than shared amongst all bacterial species.

B219

Klebsiella pneumoniae triggers a singular macrophage polarization to promote infection

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Abstract

Klebsiella pneumoniae (*K.p*) is a Gram-negative bacterial pathogen responsible for nosocomial and community-acquired infections including devastating necrotising pneumonia. Indeed *K.p* is an important secondary infection in relation to COVID-19 patients. Treatment options are rapidly diminishing as "last resort" antimicrobials are becoming ineffective. Therefore, better understanding of *K.p* pathogenesis has never been more urgent. As the first line of cellular response to infection, macrophages play an integral part in control of *K.p.* Herein, we demonstrate in detail that *K.p* can actively redirect macrophage polarisation for its own benefit, provide evidence for myeloid cells involved in this process and have delineated the cell signalling mechanism involved in full. .

Given the highly plastic nature of macrophage polarisation, it is no longer adequate consigned to one of two broad subgroups. Using scRNAseq, flow cytometry and Seahorse assays, we have successfully described what we term M2*kp* macrophages in detail. Via *in vitro* analysis we were able to discern that *K.p* induces and M2*kp* response via TLR4-Myd88-TRAM/TRIF-STAT6- PPARg pathway. We were able to reverse this phenotype via inhibition of STAT6 signalling *in vivo* leading to bacterial clearance in mice. Key features of M2*kp* were repeated in human macrophages and previously in porcine tissues and cell lines, inferring this robust response is ubiquitous across three species. In conclusion we have identified a novel mechanism by which *K.p* averts destruction and a method to utilise host immunity to counteract it- a highly important issue as we approach a post-antibiotic era.

B220

Interactions of adherent-invasive *Escherichia coli* with human intestinal epithelia

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Abstract

Background

Crohn's disease is a chronic intestinal disorder affecting more than 120,000 people in the UK. Previous studies have shown a prevalence of adherent-invasive *Escherichia coli* (AIEC) in Crohn's biopsy tissues. AIEC invade and survive in non-confluent human intestinal epithelial cells but their interaction with confluent epithelia has not been investigated.

Methods

Confluent Caco-2 and T84 colon carcinoma cells were incubated with AIEC strain LF82 or non-invasive *E. coli* MG1655. Adhesion, invasion and intracellular replication were determined by quantifying colony-forming units (CFUs). Gentamicin was added to kill extracellular bacteria. Host cell integrity, AIEC receptor expression (CEACAM-6), and adhesion was evaluated by immunofluorescence staining.

Results

Quantification of CFUs showed that LF82 adherence, invasion and replication was significantly higher compared with MG1655. This was more pronounced in Caco-2 versus T84 cells. Notably, LF82 invasion was considerably lower than that reported in non-confluent Caco-2 and T84 cells. Fluorescence microscopy indicated loss of integrity in LF82-infected T84 but not Caco-2 cells. Both cell lines heterogeneously expressed CEACAM-6, but receptor distribution did not correlate with LF82 binding. Surprisingly, LF82 adhesion to both cell lines was much more pronounced than expected from CFU results. Immunostaining indicated the formation of biofilms containing dormant AIEC.

Conclusion

Our results suggest AIEC pathogenesis in confluent intestinal epithelia is determined by extensive biofilm formation rather than invasion as demonstrated for non-confluent cells. AIEC dormancy within biofilms is likely to promote immune evasion and antibiotic resistance. The relevance of these findings will be confirmed in future studies using human intestinal organoids.

B221

Discovery and investigation of the C13 legumain-like protease in *Acinetobacter baumannii*

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Abstract

Acinetobacter Baumannii is a gram-negative bacterium whose nosocomial transmission rates and combined ability for antimicrobial resistance have made it a pathogen of high interest in recent years. A recent inhibitor screen showed that several cysteine protease inhibitors, particularly a legumain-specific inhibitor, were effective in reducing *A. baumannii* biofilm formation. This was unexpected as the C13 protease family, of which legumain is the main member, have been poorly characterised outside of plants, humans, and helminths where they are generally found in acidic vacuoles. Indeed, many bacteria do not even possess these proteases.

However, a MEROPs database screen confirmed the presence of a single C13 family member in *A. baumannii* leading us to investigate if this was the target of the inhibitor. To investigate this legumain-like protein (LLP), we cloned its C13 protease domain into an expression construct for *Escherichia coli* and examined its activity against a legumain-specific substrate. Upon confirmation of legumain-like enzyme activity, cysteine inhibitors used in the previous screen for *A. baumannii* biofilm reduction were tested, and reduced the enzyme activity observed.

Further studies are currently underway to investigate the potential contribution of this C13 protease to *A. baumannii*'s antibiotic resistance in both planktonic and sessile cell forms.

B222

Identification of potential vaccine candidates against *Acinetobacter baumannii* using a novel wet-dry approach

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Abstract

Multi-drug resistant *Acinetobacter baumannii* is prevalent worldwide as a predominant cause of nosocomial infections, imposing serious burdens on global healthcare systems. To date, there are no licensed vaccines against it. Previous efforts to identify vaccine antigens based on experimental or computational approaches were quite laborious. Here we developed a novel approach combining both wet- and dry-lab technology to identify potential vaccine candidates against *A. baumannii* more effectively.

The unique proteomic platform 'Cell Blot' developed in our laboratory was used to identify *A. baumannii* adhesins involved in host-cell attachment. This technology probes bacterial proteins with human epithelial cells before detecting the attached cells with specific antibodies and identifying adhesins by LC-MS. Proteins identified were further analysed with bioinformatic tools for homology, conservativeness, structure, epitopes and antigenicity prediction. A score-based selection process following the logic of reverse vaccinology was designed to shortlist candidates with the most vaccine potential.

Previously reported immunogenic *A. baumannii* proteins were identified with this approach (including Omp38, Omp33-36, OmpW, Omp22, CarO and BAM subunits), in addition to several novel candidates. Specifically, seven very promising novel antigens were consistently identified in the wet-lab cell blots and were predicted *in silico* to be highly conserved, non-human homologous and highly antigenic. These are being cloned and expressed for validation and to evaluate their potential in mouse immunisation and challenge studies.

In summary, this study offered a novel approach which, once further validated, could be developed into an optimised platform for quick and reliable antigen identification in modern vaccine development.

B224

The pangenome of *Fusobacterium nucleatum* subsp. *polymorphum* recovered from potentially malignant oral leukoplakia

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Abstract

Fusobacterium nucleatum is an anaerobic commensal of the oral cavity, associated with periodontitis and extra-oral diseases, including colorectal cancer. Previously, our studies have shown an increased relative abundance of this bacterium on potentially malignant oral leukoplakia (OLK). Using direct culture, we found that 79% of *Fusobacterium* species isolated from OLK patients were *F. nucleatum* subsp. *polymorphum*. Whole genome sequencing (Illumina and MinION) and pangenome analysis with Roary was carried out on 75 isolate genomes, including 59 genomes sequenced in this study and 16 genomes recovered from GenBank. This analysis has shown the core genome shared by these isolates is relatively small compared to the large accessory genome, with many individual isolates possessing genes unique to them. A phylogenetic tree produced by comparing the core genome shows the isolates can be separated into three main clades. Isolates from healthy and OLK sites of the same patient cluster together, indicating they are genetically highly related. A large repertoire of adhesins belonging to the Type V secretion system could be identified. However, there is variation in the copy number of the major adhesins, such as FadA and Fap2 among isolates. Variation is also seen in phenotypic tests, with differences seen in the isolates' ability to hemagglutinate red blood cells and resist killing by human serum. Overall, it appears that although *F. nucleatum* subsp. *polymorphum* isolates from the same patient are genetically similar, the collection shows variability in genotype and phenotype, indicating potential variation in virulence in the population.

B225

Interactions of clinical *Streptococcus pyogenes* of different genotypes with human tonsil

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Abstract

Streptococcus pyogenes, also known as Group A *Streptococcus* (GAS) is a human pathogen associated with a wide array of infections. Human tonsils are commonly the primary site of *S. pyogenes* infection, however limited knowledge of this interaction exists due to lack of appropriate infection models. The ability to infect human tonsil is likely to be key to the emergence of successful new variants that become dominant in the population. The most recent being the new genotype *emm*89 variant, that emerged following recombination resulting in the loss of the hyaluronic acid capsule and increased levels of the toxins NADase and streptolysin O.

We assessed the ability of clinical isolates belonging to 18 of the most common *S. pyogenes emm*-types, for which we have whole genome sequence data, to adhere and invade primary human tonsil keratinocytes. While all isolates were able to adhere and invade, the ability of these to do so varied by *emm*-type. Isolates of the new variant of *emm*89 consistently showed greater adhesion/invasion compared to the old *emm89* variant. Further work using a novel tissue-engineered 3D tonsil model, which models the epithelium and underlying tissues of native human tonsil, supported the findings that the new variant is better able to infect and survive in this key site.

Our human tonsil infection models are a unique research tool for delineating the mechanisms of primary *S. pyogenes* infection. Work is on-going to explore the role of the capsule and the NADase/streptolysin O toxins, and other virulence factors in this setting.

B226

Non-O157 Shiga toxin *Escherichia coli* serogroups (STEC) in Scottish cattle: diversity and virulence gene attributes

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Abstract

Shiga toxin (*stx*)-producing *Escherichia coli* (STEC) are zoonotic pathogens carried by ruminants that can cause serious human disease. In this study we examined the diversity of non-O157 serogroups and virulence profiles in a collection of *stx*-positive and -negative strains (n=115) derived from 53 Scottish cattle herds sampled between 2014 to 2015. Strains were isolated following culture on CHROMagar^{TM} STEC selective media and pair-end sequenced using the Illumina Miseq, with O:H serotype determined by the Scottish Microbiology Reference Laboratory (SMIRL) Edinburgh WGS Bioinformatics Workflow. Genomes were screened for 2701 virulence genes using the Virulence Finder Database. A Maximum Likelihood phylogeny was constructed by core genome alignment.

Twenty different non-O157 *stx*-positive serotypes were identified in 31 herds, of which 10/20 included *stx1*-positive and 13/20 *stx2*-positive strains. All dual *stx*- and *eae*-positive serotypes (8/20) carried core virulence genes *ehxA*, *tir*, *tccP*, *espA*, *espF* and *nleB* and were identified as O26:H11, O150:H2, O10:H25, O145:H28, O177:H11, O182:H25, O84:H2 and O98:H21. Excluding O26:H11, the most frequently identified STEC was the e*ae*-negative O136:H12 serotype (six herds), followed by O182:H25 (three herds). Overall, 4 serotypes met the criteria for JEMRA classification level 1, based on virulence characteristics, indicating the highest pathogenic potential to humans. Multiple STEC carriage was identified in nine herds, with one herd yielding five different STEC serotypes. Mixed carriage of *stx*-negative and *stx*-positive strains occurred in both samples and herds. These data indicate that cattle herds in Scotland harbour a diverse range of pathogenic STEC which are of potential concern for public health.

B227

Experimental Evolution of Pseudomonas aeruginosa in media replicating the conditions of the upper and lower airways

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Abstract

Cystic fibrosis affects >70,000 people worldwide and *Pseudomonas aeruginosa* (Pa) is the predominant pathogen of the CF lung. During early infection, Pa adapts to the respiratory environment and drivers of adaptation to the lung are poorly understood but the sinuses are thought to act as a site of early colonisation within which adaptation to host can occur. There is a need for more robust *in vitro* models that reflect the environmental conditions of the CF airways, for use in CF pathogen virulence screening and for study of microbial evolution.

The respiratory tract is a heterogenous environment, with the sinuses and lung having distinct properties. However, currently available *in vitro* models focus on the lung environment, with less consideration given to upper airway environments.

We have developed culture media reproducing key features of the upper and lower airways for the study of bacterial evolution and virulence. We used this media to perform experimental evolution with Pa grown in sinuses like media (SLM) and lung like media (LLM) under conditions reflecting either health or CF. I will present preliminary data from this work, defining the composition of the media and describing observed phenotypic and genotypic changes in Pa experimentally evolved in SLM and LLM. Pa evolved under CF conditions underwent changes typically associated with clinical isolates from CF. These novel media offer a unique opportunity to study how microbial adaptation to upper airway environments influences subsequent colonisation of lung and establishment of chronic infection.

B228

MspA: a scaffold for lipotechoic acid synthesis in *Staphylococcus aureus*?

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Abstract

Staphylococcus aureus is a major human pathogen that can colonise the host asymptomatically but also cause invasive infections. *S. aureus*' cell wall and membrane are critical to its infection capabilities. Lipoteichoic acid (LTA) is a cell wall polymer synthesised by the enzymes UgtP, LtaA and LtaS, that is anchored to the membrane and implicated in cell division, autolysis and resistance to cationic antimicrobial peptides (CAMPs). Membrane Stabilising Protein A (MspA) is a small protein evenly distributed across the *S. aureus* membrane, the inactivation of which has pleiotropic effects on virulence, including toxin production, membrane stability, susceptibility to fatty acids and CAMPs, and iron homeostasis. However, the mechanism whereby MspA affects these phenotypes is still unclear. Electron and fluorescence microscopy suggest that *mspA* mutant cells are on average larger than wild type cells, a feature shared by *ypfP* and *ltaA* mutants. A bacterial two-hybrid system confirmed that MspA interacts with the three enzymes of the LTA pathway, whereby the *mspA* mutant had higher amounts of LTA associated within the cell envelope. qRT-PCR also demonstrated that *ltaA*, but not *ltaS*, is overexpressed in the *mspA* mutant. We hypothesise that MspA could be acting as a membrane scaffold stabilising the protein complex formed by UgtP, LtaA and LtaS, where in the absence of MspA, the complex would be perturbed, leading to increase production of LTA as a compensation strategy. Further experiments are underway to determine whether these interactions underly the means by which MspA has such pleiotropic effects on the pathogenicity of *S. aureus*.

B229

Investigating the Adaptations of *Pseudomonas aeruginosa* to Hypoxia

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Abstract

Pseudomonas aeruginosa is a ubiquitous and opportunistic pathogen which causes chronic infections thus reducing the expectancy and quality of life of people with debilitating illnesses like cystic fibrosis (CF). Indeed, the leading cause of death in people with CF is lung disease due to chronic infection. *P. aeruginosa* is a multi-drug resistant organism and consequently eradication is near impossible. The hallmarks of the chronically colonised CF lung are chronic inflammation, lung cell damage, lung function decline and hypoxia. This study examines the impact of hypoxia on clinical isolates of *P. aeruginosa* and identifies proteins associated with its adaptation to a hypoxic environment. Two early CF infection isolates, AA2 and AMT0060.3, were characterised in terms of motility, biofilm formation, and growth. Strain AA2 was cultured in hypoxic and normoxic conditions for 72hrs with periodic sampling to monitor survival and adaptation by proteomic analysis using mass spectrometry. Colony counts indicated successful adaptation of AA2 to hypoxia within 12hrs as determined by a 26.5% increase in viable cells. No effect of hypoxia on motility was observed ($p > 0.05$). Extensive biofilm formation was observed in the hypoxia-adapted strain but not the normoxia comparator (18-fold difference), along with previously unreported pyorubin synthesis. Proteomic analysis revealed increased abundance of OprI, an outer membrane protein, and the reduced abundance of hisZ, a regulatory subunit of the histidine biosynthesis pathway which has been implicated in oxygen sensing, in the hypoxia-adapted replicates. Further elucidation of these adaptations will allow us to target this pathway effectively.

B230

Drosophila versus *Mycobacteria*: Using host-pathogen interactions to study physiology and pathogenesis of *Mycobacterium abscessus*.

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Abstract

Mycobacterium abscessus is a highly resistant emerging pathogen which poses a major threat to susceptible individuals, particularly those with Cystic Fibrosis. *M. abscessus* shares some pathogenic traits with other mycobacteria such as *M. tuberculosis* and has also acquired non-mycobacterial virulence factors from species such as *Pseudomonas aeruginosa*. Little is known about the infection process and intracellular behaviours used by *M. abscessus* to survive and proliferate within the hostile intracellular environment, nor about the innate host immune responses that may detect and respond to *M. abscessus* infection and how these host responses impact the behaviour of this bacterium. 18 *M. abscessus* genes were targeted for knockout, with target genes selected based on a previous GWAS that identified *M. abscessus* genes associated with infection phenotypes, homology to known tuberculosis virulence factors, and potential involvement in host-pathogen metabolic interactions. These mutants were then characterised using a *Drosophila melanogaster* in vivo infection model, providing insight into the role of each of these *M. abscessus* genes in infection and intracellular survival. So far, 10 of these mutants have shown a defect in *in vivo* infection based on *D. melanogaster* survival and bacterial load during infection. In the immediate future, we intend to exploit the genetic tractability of *D. melanogaster* to define the genetic architecture of the interaction between host and pathogen revealed by these bacterial mutants.

B231

High incidence of resistance to antibiotics used in the eradication of *Helicobacter pylori*

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Abstract

Helicobacter pylori is the leading cause of peptic ulcers and gastric cancer. Treatment is becoming more problematic due to increasing antibiotic resistance rates globally. The WHO designated clarithromycin-resistant H. pylori as a high priority pathogen for drug development. There is no routine surveillance of H. pylori antimicrobial sensitivities in the UK, and published data is lacking. We recently found that 10% of patients required at least 3 rounds of therapy for successful eradication. This study aimed to characterise antimicrobial sensitivities of isolates collected in Nottingham since 2001.

Gastric biopsy samples were collected, with informed written consent and ethics approval, from patients attending the Queen's Medical Centre in Nottingham for an upper GI tract endoscopy. Antibiotic sensitivity was assessed using disc diffusion and Etest methods, with strain NCTC11637 as a control.

Of 241 isolates tested, 28% were resistant to clarithromycin, 68% to metronidazole, and 3% to amoxicillin, which are used in first-line therapies. For those used in second- and third-line therapies, 0% of isolates were resistant to tetracycline, 4% to levofloxacin and 13% to rifampicin. Multi-drug resistance was found in 33% of isolates. Resistance to clarithromycin increased dramatically between 2001-2005 and 2011-2018 (17% to 45%; p=0.04).

Resistance rates were higher than had previously been estimated for UK isolates. Based on the resistance profiles, treatment failure is more likely to occur when patients are given first-line therapies without amoxicillin. We are now sequencing the genomes of the levofloxacin-resistant isolates, to gain insight into the relationship between genotypic and phenotypic resistance data.

B234

The effects of environmental stresses on *Campylobacter jejuni* biofilm formation

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Abstract

Campylobacter jejuni is a commensal of the chicken microbiome that is a common foodborne pathogen in humans. Some isolates of C. jejuni have been shown to have the ability to form biofilms but significant variation has been seen between different strains. Biofilms can allow the bacteria to persist long term on supermarket chickens, increasing the chance of human consumption and subsequent infection. Other pathogens have been shown to display increased biofilm formation in the presence of environmental stressors such as salt, glucose concentration, and ethanol. The aim of this study is to identify and measure the effects of environmental stresses on biofilm formation by multiple fresh isolates of Campylobacter jejuni. Crystal violet biofilm assays were used in the presence of different concentrations of multiple environmental stressors. Differences in growth rates and biofilm production were assessed under both microaerobic and aerobic conditions to assess the impact of environmental stresses on the survival of C. jejuni in the supermarket environment.

B235

Shiga-toxin producing *Escherichia coli* clonal complex 32, serotype O145:H28 in the UK

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Abstract

STEC serotype O157:H7 was the causative agent of outbreaks of Haemolytic Uremic Syndrome (HUS) when STEC first emerged in the 1980's. Over the last decade we have seen a decrease in STEC O157:H7 and an increase in non-O157 serotypes, such as O145:H28. Isolates of O145:H28 in the archives of public health agencies in the UK were whole genome sequenced, virulence profiled and integrated with enhanced surveillance questionnaire (ESQ) data including exposures and disease severity.

Until 2021, there were 290 diagnosis of STEC O145:H28 in the UK and Ireland, of which 268/290 had *stx2a*, the *stx* subtype most commonly associated with STEC-HUS. The majority of cases were female (61%), and aged 0-4 years. In England, ESQ data was available for 106/118. Diarrhoea was the most common symptom (92%) followed by abdominal pain (84%), blood in stool (63%). Thirteen cases developed HUS, and one child died.

Genomic typing provided evidence of household transmission and small, temporally related, geographically dispersed outbreaks, most likely to be foodborne. Although the source and transmission routes for STEC O145:H28 are yet to be established, genomic and epidemiological analysis indicates that they are similar to STEC O157:H7.

Inconsistencies within the UK regarding testing strategies for STEC O145:H28 mean determining true incidence, prevalence and transmission routes is challenging. The reported disease severity provided evidence that STEC O145:H28 is a clinically significant pathogen and an emerging threat to public health. Improved diagnostic capabilities and surveillance strategies are required to ensure outbreaks of STEC O145:H28 can be detected and investigated.

B237

Epidemiology of protozoan and helminthic parasites in wild passerine populations of the British Isles

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Abstract

Avian endoparasites play important roles in conservation, ecology, biodiversity, and host evolution. There is a lack of current epidemiological studies on the frequency and dispersal of intestinal parasites infecting wild passerine birds, which are necessary for understanding host-parasite dynamics. The objectives are to determine the occurrence and distribution of helminths and protozoans parasitising various avian hosts as well as to identify the spatiotemporal variation of infection biogeography; subsequently, ecological factors of feeder presence as well as host habitat and diet diversity were examined to determine whether these variables explained any patterns observed. Faecal samples (n = 755) from 38 bird species, representing 18 Passeriform families, were collected from 7 sites across England, Wales, and Ireland from March 2020 – June 2021. A conventional Sodium Nitrate flotation method allowed morphological identification and abundance estimation of eggs and oocysts. Overall, 20.0% of specimens were positive with Corvidae, Prunellidae, and Sturnidae having the highest infection rates while six families remained uninfected; meanwhile, Fringillidae and Emberizidae had the highest and lowest amounts of faecal parasite counts in positive samples respectively. Moreover, *Syngamus*, *Isospora*, and *Capillaria* were the most prevalent genera observed in positive samples. The presence and abundance of intestinal parasites were found to significantly differ amongst avian host families as well as season and geographical region; phylogenetically controlled models confirmed the significance of spatiotemporal variation. Amongst ecological factors, only host diet diversity was found to be significantly associated with parasite abundance. The effects of endoparasitism on these wild passerine populations will then be discussed.

B238

Genome-scale analysis of *Staphylococcus aureus* to identify determinants of avian host-adaptation

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Abstract

Staphylococcus aureus is a major human and animal bacterial pathogen that causes an array of diseases, including osteomyelitis and pododermatitis in chickens. Avian *S. aureus* originated from human-to-chicken host-jump events, followed by host-adaptation via gene acquisition and diversification. Our research aims to combine transposon (Tn) mutagenesis of *S. aureus* with *ex vivo* experimental infections to identify the immune cell repertoire and bacterial genes involved in avian host-pathogen interactions and adaptation. To determine the avian immune cell tropism for *S. aureus*, mCherry-integrated clones from common avian (CC385, CC5) and human (CC8) *S. aureus* clonal lineages were screened in blood extracted from the transgenic chicken line Runx1-eGFP. Staining with GM-CSF1 allowed visualisation of the avian peripheral blood leukocyte (PBL) populations; monocytes, heterophils, basophils and eosinophils. We demonstrate that monocytes and heterophils generate the first-line response to *S. aureus* infection, with avian strains exhibiting differential uptake by heterophils compared to a human strain. Furthermore, our analysis suggests that this adaptation of avian *S. aureus* strains may involve inhibition of degranulation of heterophils as a survival mechanism; further work characterising this process is underway. To identify all the *S. aureus* genes required for bacterial survival and immune evasion we are currently employing the first avian *S. aureus* Tn-mutant library in experimental infection of avian PBLs. Overall, this study provides new insights into the evolution of avian-adapted *S. aureus* and the key host-pathogen interactions underpinning *S. aureus* infections of chickens.

B239

A rare case of *Aerococcus urinae* infective endocarditis: case report and literature review.

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Abstract

Introduction

Aerococcus urinae is emerging as a highly virulent pathogen capable of invasive infection, typically in older males with underlying urinary tract co-morbidities. A. *urinae* endocarditis is rare; since 1995 fewer than 50 cases have been described in the literature.

Case Report

We present a case of *A. urinae* infective endocarditis, successfully treated with mitral and aortic valve replacement, followed by a prolonged antimicrobial course.

Our patient, a 65-year-old male with previous good health, presented with flu-like symptoms. Rapid clinical deterioration ensued with the development of acute pulmonary oedema requiring invasive ventilation. During routine urinary catheterisation, a urethral stricture was discovered.

A. urinae was isolated from a peripheral blood culture and an echocardiogram demonstrated aortic and mitral valve endocarditis, massive vegetations and suspected aortic root abscess.

Accompanying features included cerebral and splenic emboli, disseminated intravascular coagulation and acute kidney injury.

Our patient was urgently referred to the regional tertiary centre and underwent bi-valvular replacement. A. *urinae* was cultured from each native valve.

Discussion

A. urinae is not usually associated with severe infection. In predisposed patients it may be responsible for invasive urosepsis, infective endocarditis and spondylodiscitis. Disease is associated with systemic embolisation and formation of biofilm, making management challenging.

Conclusion

Identification of *A. urinae* in blood cultures should prompt urgent investigation to exclude endocarditis. Use of newer technologies such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-

TOF) mass spectrometry assists early identification, prompting appropriate investigations and timely initiation of aggressive antimicrobial therapy and surgical intervention where appropriate.

B240

Evidence of a link between fluoroquinolone resistance and aerobic growth in freshly isolated strains of *Campylobacter*.

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Abstract

Campylobacter jejuni is the leading cause of bacterial diarrhoea worldwide. The consumption of contaminated chicken meat is the most common route of infection, but how this microaerophilic pathogen survives within the aerobic supermarket environment remains unclear. Recent work carried out in our lab suggested a possible link between aerobic survival and fluoroquinolone resistance. However, other studies have identified strains of *C. jejuni* which are capable of aerobic growth. The aim of this study was to investigate whether growth under aerobic conditions was also associated with fluoroquinolone resistance using a panel of freshly isolated strains.

An association between fluoroquinolone resistance and aerobic growth was observed as isolates which displayed high levels of resistance to fluoroquinolone antibiotics exhibited an enhanced ability to grow aerobically. The ability of strains to grow aerobically could also be increased by serial passage under aerobic conditions which in turn increased the isolates' level of resistance to fluoroquinolones. In addition, the acquisition of fluoroquinolone resistance by sensitive strains, 81-176 and 11168 following passaging at sub lethal concentrations of antibiotics, led to an increase in aerobic growth.

These results demonstrate a clear link between aerobic growth and fluoroquinolone resistance. This link is supported as the selective pressures of aerobic stress or fluoroquinolone antibiotics could generate increased antibiotic resistance or aerobic growth respectively. This work supports our previous data linking aerobic survival and fluoroquinolone resistance and suggests that the aerobic supermarket environment may be selecting for fluoroquinolone resistant strains within the food chain.

B241

Staphylococcal type VII effector interactions with host pathways

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Abstract

Staphylococcus aureus, a major hospital and community associated pathogen, causes infections ranging from impetigo to bone infections, which are attributed to the wide range of virulence factors it possesses. One such virulence- associated system is the type VII secretion system (T7SS), which exports several effectors including EsxA. EsxA is a WXG-100 family helix-turn-helix protein, that is required for virulence in murine infection models. EsxA has been implicated in modulating host epithelial cell apoptosis during intracellular infections, although the underlying mechanisms are unclear. The aim of this study is to understand how EsxA is involved in modulating epithelial cell apoptosis. We first demonstrated EsxA can dely apoptosis in other epithelial cells like MG-63 bone fibroblast in addition to lung cells. To identify if EsxA interacts directly with host factors mediating modulation of apoptosis, the host protein interaction partners of EsxA in epithelial cells was explored using proximity-based biotin identification method (BioID).We tagged *esxA* with *birA*, which encodes an enzyme that biotinylates protein/s that comes in proximity of the protein of interest. Tagged constructs were expressed in epithelial cells followed by purification of biotinylated proteins with streptavidin beads and liquid chromatography mass spectrometry (LC-MS). We identified 40 proteins in *esxA*-expressing cells compared to controls, that included some protein involved in programmed cell death. One potential candidate, 14-3-3 epsilon, which is indicated to bind to EsxA by computational modelling, is being confirmed by immunoprecipitation experiments. Thus, our data indicates that the T7SS may interfere in host cell death through effector interactions.

B243

Effects of Low-Intensity Pulsed Ultrasound (LIPUS) on S. aureus Biofilms

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Abstract

Low-intensity pulsed ultrasound (LIPUS) has current therapeutic applications, including in fracture clinics to aid bone healing. Research into other potential uses has found that LIPUS increases antimicrobial sensitivity in some bacterial species including Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. The mechanisms behind this have not yet been elucidated. Chronic skin infections are often associated with microbial biofilms; biofilms are present in more than 60% of chronic infections, compared to 6% of acute infections. These chronic biofilm infections are difficult to treat due to increased antibiotic tolerance exhibited by biofilm associated bacteria. This tolerance results in extended and repeated antibiotic use, and poor antimicrobial stewardship increases risk of the development of antimicrobial resistance. The aim of this project is to establish whether LIPUS reduces antimicrobial tolerance of S. aureus biofilms, investigate how biofilm maturation impacts effects of LIPUS on antibiotic tolerance, identify mechanisms by which tolerance is altered and to use a tissue-engineered infected skin model to identify potential for real world clinical application. Results to date indicate that treatment with LIPUS may alter the structure and permeability of S. aureus biofilm as well as reducing biofilm tolerance to gentamicin treatment. LIPUS treatment may aid in antibiotic stewardship by reducing amounts and frequencies of treatment needed to treat chronic infection.

B244

Investigating the cause of sodium hypochlorite (NaOCl) resistance in clinically relevant Clostridioides difficile strains.

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Abstract

Clostridioides difficile is a spore-forming obligate anaerobe found to colonise 1-5% of adults as part of the normal colonic microbiota. This microbiota is the first line of defence against *C. difficile* infection (CDI), thus upon dysbiosis, vegetative *C. difficile* may proliferate and become pathogenic resulting in toxin-mediated CDI. Characteristic diarrhoeal symptoms result in the release of ~1×10^7 highly resistant *C. difficile* spores into the environment, propagating transmission and resulting in CDI as a significant cause of hospital acquired infections. Proper cleaning and disinfection strategies within healthcare settings are vital to decrease infection rates. The current recommendation for spore deactivation requires 1000ppm available chlorine for 10 minutes; however, evidence suggests this strategy is not sufficient to fully deactivate *C. difficile* spores. Consequently, it is important to review the sporicidal efficacy of NaOCl at recommended concentrations and to establish whether viable *C. difficile* spores are recovered after disinfection, and determine phenotypic or genotypic resistance indicators. In this study, clinically relevant CD630 and R20291 *C. difficile* strains were exposed to varying NaOCl concentrations to examine spore viability and potential morphological changes via transmission electron microscopy. PCR and sequencing methods were utilised to identify any genetic changes that may confer biocide resistance, with a focus on *CotE* and *CdeC* genes. R20291 spores demonstrated high phenotypic resistance following serial NaOCl passage treatment above recommended concentrations, with visible changes to the exosporium. This information is vital to review current disinfection strategies in the fight against nosocomial CDI in this antimicrobial era.

B246

Identification of novel *Pseudomonas aeruginosa* vaccine antigens using an innovative proteomic approach

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen with great adaptability. It causes severe infections for immunocompromised individuals and patients with cystic fibrosis (CF), bronchiectasis or chronic obstructive pulmonary disease (COPD), usually establishing chronic infections that lead to poor prognosis and lung function decline. Due to its antimicrobial resistance (AMR) mechanisms, drug therapy is often ineffective. Therefore, a vaccine that prevents infection would improve susceptible patients' quality of life and reduce both mortality and the spread of AMR. Because bacterial adhesins have shown good efficacy as vaccine antigens, we aim to identify proteins involved in *P. aeruginosa* attachment to human lung epithelial cells (16HBE14o^{-}), which are potential effective antigens for a prophylactic vaccine. The ability of six clinical *P. aeruginosa* isolates from different sources of infection to attach to 16HBE14o^{-} cells was shown to be strain-dependent by two methods (colony counting and confocal microscopy). A CF transmissible strain (LES 431) showed the lowest level of attachment, followed by a burn isolate (Mi 162). Strains from COPD and CF patients (57P31PA, AA2, ATM 0060-3) showed moderate attachment, while the community acquired pneumonia isolate (A5803) showed the highest attachment. Using our 2D cell blot method and mass spectrometry, we identified seven novel adhesins common to at least 2 strains. These have the potential to be effective vaccine antigens as they are surface-exposed, predicted to be immunogenic, and have low homologies with human, mouse or *Escherichia coli* proteins. Their protective potential will be examined in immunisation and challenge studies in mice.

B247

Manuka Honey: A potential treatment for respiratory infections in cystic fibrosis patients

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Abstract

Cystic fibrosis is a condition that affects 1 in 2500 people, and one of the most problematic symptoms is the inability to breakdown mucus within this lungs. The development of chronic respiratory problems is exacerbated by bacterial infections. Multi-drug resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) is a one of the primary causes of chronic respiratory infection in cystic fibrosis patients. Manuka honey has shown activity against *P. aeruginosa* in wound infections but has yet to be studied for respiratory infections.

EUCAST broth microdilution was used to determine the minimum inhibitory concentration of manuka honey and standard of care antibiotics tobramycin and ceftazidime against two strains of *P. aeruginosa*. Antibiotic checkerboards were used to identify interactions between manuka honey and the antibiotics.

Once inhibitory concentrations of antibiotics and honey were determined those concentrations were applied to human lung epithelial cell line A549 to determine any cytotoxicity. The production of IL-8 from A549 cells that had been infected with *P. aeruginosa* and treated with manuka honey, tobramycin, ceftazidime for 2 hours was monitored via ELISA.

The minimum inhibitory concentration for manuka honey was 10-20% w/v, 16 µg/ml for tobramycin and 2-4 µg/ml ceftazidime. Synergistic interactions were seen between manuka honey and ceftazidime against PAO1. Treatment of A459s with antibiotic or honey did not cause cytotoxicity but treatment of infected A459s with manuka honey did significantly p value >0.05 Reduce IL8 production.

These results indicate that manuka honey has potential to treat *P. aeruginosa* respiratory infections and work synergistically with some antibiotics.

B249

Substrate recognition by the Legionella pneumophila type II secretion system

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Abstract

Many gram-negative bacteria use a type II secretion system (T2SS) to transport proteins out of the cell and in pathogenic strains this is a major virulence factor that can drive infection. In recent years substantial progress has been made in our understanding of the overall structure and assembly of this device but the mechanism by which its cargo is recognized and then how this triggers their export remains unclear. *Legionella pneumophila* is an opportunistic gram-negative bacterium and is the causative agent of Legionnaires' disease, an often-fatal pneumonia. *L. pneumophila* secretes between 25-60 type II substrates; the largest known catalog and with the highest number of published experimental structures of any T2SS. As these are thought to be recognized through a structured 'conformational' motif on their surface, the *Legionella* T2SS is a model system to study cargo selection. Two T2SS proteins LspC (GspC) and LspD (GspD/secretin) connect the inner and outer membranes through their HR and N0 domains, respectively. Using nuclear magnetic resonance (NMR) spectroscopy, we have determined the structures of LspC and the LspD N0 domain, have identified and characterised binding between LspC and LspD and the substrate NttA, and have derived experimental models for these complexes. In addition, we have assessed binding of other substrates to LspC and LspD and observe a range of affinities. Our data provides new insight into substrate recognition by the *L. pneumophila* T2SS but also suggests that different cargo may be selected using different mechanisms.

B251

Development of a copper antimicrobial coating on pre-existing medical devices

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Abstract

Acinetobacter baumannii is an increasingly antimicrobial resistant bacteria species that is a prevalent cause of nosocomial infections. In Europe, Eastern Mediterranean and Africa, 42 cases per 1,000 patients in ICU's recorded nosocomial *A. baumannii* infection between 2014 and 2019. Its prevalence is increasing due to increasing reliance of antibiotic usage and the increase of carbapenem resistant strains. Colistin is an antibiotic still generally effective against this species although it is reported that new colistin resistant strains are emerging. A major cause of these infections is colonisation of medical devices used in ICU's.

By using copper nanoparticles, novel coatings can be developed for medical devices such as endotracheal tubes, which will lower the risk of infection via the copper nanoparticles bactericidal properties. In this study, preliminary testing of copper nanoparticles and silver acetate which was printed onto cardboard, as a validation of their bactericidal properties on a common surface. A range of species were tested including isolates of *Acinetobacter baumannii, Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli* to examine the efficacy of the coating against both Gram-positive and Gram-negative species.

Preliminary data showed that after just one hour, there was a significant reduction in viability, in all species except *E. coli* on the copper treated cardboard. By investigating the properties of copper nanoparticles against a range of strains and using different coating techniques, pre-existing medical devices can be treated to reduce the incidence and severity of nosocomial infection.

B252

PSEUDOTYPE VIRUSES: TOOLS TO ADDRESS EMERGING ZOONOTIC THREATS

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Abstract

COVID-19 has highlighted the connection between human and animal health and our shared environment. This constantly changing human-animal-environment interface has resulted in the spread of both existing and new or emerging zoonotic diseases. More than 60% of emerging infectious diseases over the last years have been zoonotic and dangerous global threats, but have not been, until recently, a top priority. The "One Health" approach, a collaborative effort across multiple disciplines, aims to change this. We now know that many of these diseases are caused by viruses originated from bats, such as Ebola, Marburg, SARS-CoV, MERS-CoV, Nipah (NiV) and Hendra (HeV), while influenza has natural avian reservoirs. Spillover of these viruses from their natural hosts to intermediate animal hosts is thought to be the most likely mode of human infection. Our challenge is to produce additional reliable tools to efficiently diagnose these infections and to identify and progress promising therapeutic and vaccine candidates. The advent of pseudotyped lentiviral vectors has enabled the study of viral interactions with antibodies, drugs, and host cell receptors with ease and without the need of high biosafety level facilities. We have produced and employed these virus pseudotypes (PV) for detecting virus-specific neutralizing antibodies and assessing antibody functionality in vitro. These important tools can be harnessed to meet strategic objectives that contribute to the strengthening of global zoonotic surveillance, expansion of virus prevention and control policies, and strengthening pandemic preparedness and response.

B253

In vitro efficacy of relebactam versus avibactam against *Mycobacterium abscessus* complex

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Abstract

Infections resulting from *Mycobacterium abscessus* are increasing in prevalence worldwide, with the greatest risk posed to patients with underlying respiratory conditions. Treatment for infections is difficult due to wide ranging intrinsic antimicrobial resistance, which is compounded by the existence of a range of subspecies within the *M. abscessus* complex, each with varying additional antimicrobial resistance profiles. Previously, the use of β-lactam/β-lactamase inhibitors within a combination therapy has been proposed as an effective treatment option for pulmonary *M. abscessus* infections. We have assessed the *in vitro* efficacy of two non-β-lactam based inhibitors, relebactam and avibactam, as agents against *M. abscessus* with their respective partner drugs imipenem and ceftazidime, as well as in triplicate combinations with additional β-lactam antibiotics against the *M. abscessus* complex. We have shown that the commercially available ratio of imipenem to relebactam is the appropriate ratio for bactericidal activity against *M. abscessus*, whereas the ratio between ceftazidime and avibactam is redundant, due to inactivity of ceftazidime to inhibit the bacteria. We have identified that the use of imipenem and meropenem alongside either relebactam or avibactam yield low minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for each *M. abscessus* subspecies, which are within the therapeutically achievable concentration ranges within the epithelial lining fluid of the lungs. We propose the implementation of imipenem with relebactam in place of stand-alone imipenem into the current treatment regime, alongside meropenem, as a future front-line treatment option for *M. abscessus* complex infections.

B255

Development of Antimicrobial Impregnated Sol-Gel Urinary Catheter Coatings to Prevent Uropathogenic *Escherichia coli* Infections.

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Abstract

Catheter associated urinary tract infections (CAUTI) are a significant burden on healthcare systems. Uropathogenic *E. coli* (UPEC), a major causative pathogen, form biofilms on the surface of the catheter resulting in infections which are recalcitrant to antibiotic therapy. Quorum sensing inhibitors (QSIs) t*rans*-cinnamaldehyde or furanone C-30, in combination with biocides contained within a silicon alkoxide based sol-gel coating has potential for use as an anti-infective catheter coating.

Checkerboard assays were performed to assess the synergism of QSIs in combination with biocides against planktonic and biofilm associated UPEC . Combinatorial cytotoxicity against mammalian cell lines was also determined. Elution of the antimicrobials from a sol-gel coating was determined by ICP-MS or LC-MS/MS, and antimicrobial efficacy of the coatings was assessed by disk diffusion and biofilm formation assays.

Combinations of the biocides PHMB, BAC and silver nitrate showed synergistic interactions in combination with both QSIs. Combinations of biocides and QSIs were often antagonistic with regards to cytotoxic activity against mammalian cells, requiring higher concentrations of each agent to have the same cytotoxic effect. There was rapid elution of the antimicrobials from the sol-gel and up to 100% of the available agent was eluted within one week.

The benefits of combining biocides with QSIs is the ability to reduce the concentrations required to effectively prevent biofilm formation and reduce cytotoxicity. The sol-gel formula used within this work is modifiable and in future could be optimised for more controlled release of the antimicrobials.

B256

Swine influenza virus infection dynamics in intensive pig production systems

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Abstract

Swine influenza virus (SIV) is associated with outbreaks of acute respiratory disease and contributes to chronic conditions such as porcine respiratory disease complex. Recent intensification of pig production systems poses a challenge for the control of SIV due to large population sizes and the continuous introduction of naïve animals. However, little is known about SIV infection dynamics within intensive systems or how SIV evolves under these conditions. Therefore, a longitudinal SIV surveillance study was performed on two farms comprising 3,000/4,000 sow breeding units and their associated weaning and finishing facilities. Pigs were sampled (nasal wipes, udder wipes and oral fluids) from each production stage (gilts, suckling pigs, weaned pigs and finishing pigs) at ~monthly intervals for one year and tested for the presence of SIV by RT-qPCR. Virus isolation in eggs and/or cells was performed and complete HA and NA gene sequences determined. Analysis of RT-qPCR results revealed that the virus was mostly absent from breeding and finishing units but was highly prevalent in weaned pig populations. Sequencing analysis showed that a single strain was in continuous circulation throughout the year, with pandemic 2009 H1N1 present on one weaning unit and human-like H1N2 on the other. Comparison of HA and NA sequences from different time-points identified a number of mutations that emerged during the year that may be associated with antigenic drift and immune escape. These results will contribute to an improved understanding of transmission dynamics in intensive systems and inform infection control practices.

B257

Listeria monocytogenes forms aggregates to increase host cell invasion success rate

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Abstract

Background: *Listeria monocytogenes* is a food-borne pathogen, the first site of infection is the host intestinal epithelium. In the intestine *L. monocytogenes* forms aggregates that aid in colonisation and persistence. We observed multi-bacteria invasion events, where the aggregates of bacteria were entering the host cells simultaneously.

Methods: We employed live-cell imaging approaches of deletion mutants for virulence genes of interest. We also used fluorescent protein reporters as markers for bacterial virulence gene expression upon infection of primary human cells. After 2 hours, the infection was treated with an antibiotic to kill the extracellular *L. monocytogenes*. Live cell imaging continued for 18 hours post-infection and the images were analysed at the single cell level.

Results: Although many bacteria were able to adhere to the membrane of the host cells, only a very small fraction of the total bacterial cell population were able to successfully invade the host cells. Successful invasion of host cells is linked to bacterial aggregates forming on the host cell surface, these aggregated bacteria are able to invade host cells simultaneously and increase the invasion success rate. These aggregates are induced by a secreted host cell factor and in a mutant unable to aggregate invasion success rate was significantly reduced.

Conclusions: Successful invasion of host cells is a rare event, by utilising an aggregation phenotype to adhere to host cells *L. monocytogenes* increases its successful infection rate.

B258

Will metformin be one of the rescuers in the antibiotic crisis?

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Abstract

Background

The rise in antibiotic resistance, resulting in life-threatening microbial disease, is especially problematic for vulnerable individuals like diabetic patients. Therefore, it is important to explore solutions to this crisis like using non-antibiotic antimicrobial agents such as metformin. Here we aimed to examine the antibacterial properties of the antidiabetic drug, metformin.

Methods

The effect of metformin on the growth of four species (*Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumoniae*) was determined. Its impacts on biofilm formation, protease production, motility and efflux were also investigated.

Results

Concentrations of metformin between 1,250 - 10,000 µg/mL
significantly decreased the growth of all species. Concentrations of 1,250 and 10,000 µg/mL reduced protease activity and biofilm formation in almost all the tested isolates. There was little effect on motility with a reduction in swimming in one *E. coli* isolate at 10,000 µg/mL metformin and no effect on twitching. Exposure to 10,000 µg/mL metformin caused a significant inhibition in the efflux activity in one *S. aureus* isolate.

Conclusions

Metformin shows some ability to impair both bacterial growth and factors associated with virulence, but only at high concentrations. Further investigations will be performed to identify the mechanisms of action of metformin towards bacteria, with the aim of developing more active analogues. The effects of continuous exposure on antimicrobial susceptibility and virulence are also being investigated.

Keywords

Metformin; Antibiotic resistance; Antibacterial; Virulence factors

B259

Genomic epidemiology of the livestock mastitis pathogen *Streptococcus uberis*

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Abstract

Streptococcus uberis is a leading cause of bovine mastitis globally, presenting a major economic, animal welfare, and food security issue. *S. uberis* is ubiquitous in the farm environment, highly heterogeneous, with poorly characterised epidemiology, making controlling intramammary infections caused by this bacterium challenging. This project aims to investigate the epidemiology of *S. uberis* and examine antimicrobial resistance and virulence-related genes using whole-genome sequencing. *S. uberis* from bovine (104 isolates) and ovine (18 isolates) mastitis cases were genome sequenced using HiSeq technology. Phenotypic antimicrobial susceptibility testing was also performed on the isolates. A recombination-adjusted phylogeny was constructed. Multilocus sequence type was assigned and AMR genes identified. The 122 isolates belong to 83 sequence types, of which 52 are novel. 53.3% of isolates were resistant to at least one of the eleven antimicrobials tested; resistance to clindamycin (50% of isolates), tetracycline (21.3%), and erythromycin (13.9%) were the most frequently observed. A total of twelve antimicrobial resistance genes were detected among the 122 isolates. Decreased susceptibility to at least one β-lactam was seen in 71.2% of isolates; alterations to penicillin-binding protein 2X were detected in these isolates. Several putative virulence factors were detected, many of which have potentially been acquired by horizontal gene transfer. Continued analysis will help to gain insight into the epidemiology, antimicrobial resistance, and host-pathogen interactions of this important livestock pathogen.

B260

Investigation of host-pathogen interactions for the design of effective therapeutic vaccinations against *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa chronic respiratory tract infections are important comorbidities in people with cystic fibrosis (CF), bronchiectasis and chronic obstructive pulmonary disease (COPD). *P. aeruginosa* can adapt to the host environment and multi-antibiotic resistance hinders its eradiation, thus vaccination is a potentially effective alternative to treat these chronic infections. Understanding host-bacterial interactions during chronic infections and elucidation of the host receptors for *P. aeruginosa* colonisation will inform the development of effective therapeutic vaccines. This project aims to investigate host-pathogen interactions in chronic *P. aeruginosa* infections to enable the rational design of antigen-adjuvant systems for therapeutic vaccinations against *P. aeruginosa*. The first step in bacterial colonisation is the interaction with lung cells. Consequently, we applied a novel non-biased proteomic approach which probed 16HBE14o- cell membrane proteins (resolved by 2-D electrophoresis) with the *P. aeruginosa* CF isolate AA43 and identified several *P. aeruginosa* candidate receptors using mass spectrometry. Among the identified proteins were disulfide isomerases, important in the attachment of other pathogens such as *Burkholderia cenocepacia* to 9HTEo- cells. Also, evaluation of host responses elicited against sequentially isolated *P. aeruginosa* strains (AA2, AA43 and AA44) from patients with CF showed a decrease in the stimulation of interleukin (IL)-8 between the early isolate AA2 and late isolate AA44 ($p<0.0001$), suggesting changes in host-pathogen interactions throughout the course of infection. Overall, the study of these interactions will facilitate the development of therapeutic vaccines against *P.aeruginosa*. In addition, our novel proteomic technique may represent an interesting tool for the identification of host receptors for other pathogens

B261

The roles of Staphylococcus aureus coagulase variants and their interplay with staphylokinase in blood coagulation.

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Abstract

Staphylococcus aureus is one of the leading causes of life-threatening bloodstream infections. The bacterium secrets proteins which interact with hemostasis, such as coagulases (Coa) which activate prothrombin (PT) inducing blood clotting and staphylokinase (Sak) which activates plasminogen promoting fibrinolysis. Coa allels are highly polymorphic and conserved within clonal complexes (CCs). Their N-terminal domains bind to PT while their C-terminal regions consist of varying numbers of fibrinogen-binding repeats. Different lengths of *coa* were recently associated with increased mortality in sepsis. On the other hand, *sak* is conserved and encoded within a pathogenicity island found in > 50% of isolates. The clinical relevance of Sak production remains unclear.

To investigate the roles of *S. aureus*-secreted Coa and Sak in blood coagulation we developed a model based on thromboelastometry, which measures viscoelastic properties of a blood clot and monitors the kinetics of clot formation and lysis. Citrated or native blood was inoculated with bacterial suspensions. The functions of *coa* variants were compared using strains expressing Coa of various CCs from an inducible vector pRMC2. Coa-dependent differences in clot initiation, maximal firmness and percent of lysis were identified, suggesting variants of the protein activate PT using different mechanisms. Interestingly, native blood clots formed in the presence of Coa lysed faster than clots formed natively, indicating their higher impermanence. Coa-dependent clot fibrinolysis was further increased in native blood inoculated with strains expressing Sak, suggesting a novel synergistic role for Coa and Sak-mediated clot instability. Assessment of clot formation based on thromboelastometry is a novel model for investigation of bacterial factors in blood coagulation.

B262

Bloodstream infection in critically ill patients – discerning the relevant demographic and infection characteristics

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Abstract

Bacteremia in ICU patients is a common complication, prolonging the hospital stay, increasing the medical expenditure and representing an important cause of mortality. It has been observed that the etiology of bacteremia within hospitals changes through time, and the multidrug resistant strains as causes are on the rise.

Hereby we present the cohort study evaluating factors correlating with bacteremia, and its etiology in patients. All adult patients with suspected bacteremia for whom blood cultures were ordered, were included. Patient data were distributed in two groups depending on positive or negative blood culture results. Following data were collected: demography, outcome, SIRS parameters, laboratory data (including, white blood cell count (WBC), CRP, haemoglobin, platelet count, creatinine, urea, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

We collected 546 negative and 119 positive blood samples. All detected bacteremias were nosocomial, and most common pathogens were S. epidermidis (32), P. aeruginosa (31), E. coli (7), C. parapsilosis (6), C. albicans (5) and K. pneumoniae ESBL (6). The dominant resistance mechanisms were based on extended-spectrum beta-lactamases belonging to the CTX-M family and OXA-48 in *K. pneumoniae* and VIM metallo-β-lactamases in *P. aeruginosa*. The predominant sources of bacteremia were pneumonia (20), IV catheter (20), cryptogenic (18), febrile neutropenia (15) and intra-abdominal infections (13). Empirical therapy was given to 105 patients, and was adjusted in 57 patients. Gender, creatinine, urea, ALT, CRP, lactate, oxygen saturation and heart rate differed between the two groups. There were no differences in the length of stay in hospital and survival.

B263

Further development of a 15-minute test using Glycan-functionalised latex nanoparticles for rapid identification of the 3 most common gram-negative uropathogens

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Abstract

Background:

Urinary tract infections (UTI) affect 150 million patients every year caused predominantly by Gram negative uropathogens.

We have developed NANOPLEX, a rapid-15-minute test that uses glycan-functionalised-latex nanoparticles, microscopy and image-analysis software to identify and enumerate bacteria directly from clinical urine samples.

As *E. coli, K. pneumoniae* and *P. mirabilis* are the most common uropathogens in UTI, we have developed probes to selectively identify these species at clinically relevant concentrations (10^5–10^8 CFU/ml).

Method:

Synthesis of NANOPLEX probes

A library of proprietary Glycans were conjugated to Molecular probes™ CML Latex Beads (4% w/v) using CuAAC coupling techniques.

Strain preparation

Cultures of *E. coli* BW25113, *P. mirabilis* NCTC11938, *K. pneumoniae* NCTC9154 were prepared via overnight incubation in TSB and standardised to clinically relevant bacterial concentrations (10^5–10^8 CFU/mL).

NANOPLEX assay

https://doi.org/10.1021/acsbiomaterials.1c00732

A library of glycans were screened using a single concentration (10^8 CFU/mL) and successful candidates were tested using an anonymised dataset (10^5–10^8 CFU/mL) to define accuracy parameters of each probe-strain combination.

Accuracy of the image-analysis-software is defined as the percentage of correctly classified predictions (true positive and true negative) over total predictions.

Results:

We were able to observe monotonic agglutination responses using NANOPLEX probes with *E. coli, P. mirabilis, and K. pneumoniae.* Anonymised testing successfully demonstrated the accuracy of all 3 strains above 85% at a clinically significant threshold of 10^5 CFU/ml.

Discussion:

NANOPLEX™ technology is actively being translated into a compact-hardware and single-use cartridge system to enable rapid UTI testing in point of care environments.

B264

Wnt signalling in Rift Valley fever virus infection of mosquito cells.

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Abstract

Rift Valley fever virus (RVFV) is an emerging zoonotic bunyavirus transmitted by mosquitoes and classified as a high priority pathogen by the World Health Organisation. The Wnt pathway is a highly conserved signal transduction pathway essential for cell cycle and developmental regulation, as well as host immune responses. We recently confirmed that the Wnt pathway aids RVFV replication in human cells, which concurs with studies showing that multiple viruses exploit the Wnt pathway for productive infection. Interestingly, differential expression of Wnt pathway genes has also been implicated in mosquito vector competence to other mosquito-borne viruses. We thus set out to characterise the role of the Wnt pathway in the regulation of RVFV infection in mosquito cells.

Firstly, we identified several Wnt response genes and found that RVFV infection of *Aedes aegypti* Aag2 cells led to differential expression of these response genes over time, indicating pathway induction by RVFV. We then established methods to silence and activate Wnt pathway genes in *Aedes aegypti* AF05 cells using exogenous dsRNA. Transient silencing of the Wnt response gene *toll7* resulted in a significant increase in RVFV replication. However, general pathway activation prior to infection had little impact on viral replication. To better understand the complex temporal and spatial regulation of Wnt signalling, we will generate stable cell lines either expressing a hyperactive mutant of the Wnt transcriptional co-activator Armadillo or lacking expression of Armadillo. These novel tools will provide a better understanding of Wnt signalling in insects generally and arboviral infections more specifically.

B265

Phenotypic and genotypic characteristics of microbes causing catheter-associated urinary tract infections in an Egyptian hospital

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Abstract

Surveillance of catheter-associated urinary tract infections (CAUTIs) is an essential tool of infection control programs to improve the quality of health care provided to patients. An epidemiological study of bacteria in Egyptian intensive care units showed that CAUTIs accounted for 63.8 % of total healthcare-associated infections from 2011 to 2017. However, little is known about the diversity of bacteria that cause CAUTIs in Egypt. Our aim was to amass and characterize a collection of bacteria causing CAUTIs in an Egyptian hospital. Between December 2020 and 2021, 161 clinical isolates were collected. Bacteria were isolated on different selective solid media (MacConkey agar, Eosin Methylene Blue agar, Cetrimide agar, Mannitol Salt agar) and streaked to purity. Based on phenotypic assays, eight isolates were identified as Gram-positive cocci, with the remainder representing a range of different *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus* sp.) and *Pseudomonas* spp. These isolates are being characterized phenotypically (antibiotic susceptibility, biofilm-forming ability) and genotypically (whole-genome sequencing, virulence and resistance genes, multi-locus sequence typing). Results from this study will inform development of improved treatment regimens for CAUTIs in the source hospital and potentially throughout Egypt. In addition, bioinformatics skills developed during this work will help Egyptian clinical microbiologists in microbial genomics methods.

B267

Novel antimicrobial materials: The use of antimicrobial iridium complexes against biofilms.

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Abstract

Background:

Due to the global rise of antimicrobial resistance, developing new antimicrobial agents is a priority. Metal-based complexes can be designed so as to produce singlet oxygen, a potent antimicrobial agent, when exposed to light of a specific wavelength.

Infections caused by *Staphylococcus aureus* and *S. aureus* biofilms can cause serious and chronic infection some of which are increasingly difficult to treat with antibiotics. Development of anti-biofilm agents are of vital importance.

This project examined two singlet oxygen-producing iridium (Ir) and their antibacterial activity against *S. aureus* strains.

Methods:

Three Ir-complexes were assessed for bactericidal activity against methicillin-susceptible *S. aureus* (MSSA) (ATCC 25923, SH1000 and clinical isolate, BH48) and methicillin-resistant *S. aureus* (MRSA) (ATCC 43300, USA300 and patient isolate, BH1CC).

S. aureus biofilms were grown and treated with Ir-complexes. Biofilm removal and inactivation was measured. Toxicity of complexes were assessed utilising human keratinocytes (HaCaT cells).

Results:

Both complexes showed potent inactivation against planktonic *S. aureus* (>5-log_{10} reduction in CFU/ml) after exposure to light. Bactericidal inactivation was found to be concentration dependant for both Ir-complexes. Anti-biofilm activity against mature *S. aureus* biofilms was confirmed at 50mM. Light activation for 15 minutes resulted in physical removal of 20–32% of biofilms and 24–73% reduction in metabolic activity. Each complex showed little cytotoxicity against keratinocytes at low concentrations (5mM) in both light and dark conditions.

Conclusions:

Novel Ir-complexes were shown to have potent antibacterial effect against both planktonic and biofilm-bound *S. aureus* with little cytotoxicity. Their clinical applications should be further investigated.

B268

The R1 core and its role in the pathogenesis and serum resistance of extra-intestinal pathogenic *Escherichia coli*

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Abstract

Escherichia coli is a major cause of urinary tract infections, bacteraemia and sepsis. CFT073 is a prototypic, urosepsis isolate of sequence type 73. This laboratory among others, have shown that strain CFT073 is resistant to serum, with capsule and other extracellular polysaccharide virulence factors imparting resistance. The interplay and regulation of such polysaccharides, which comprise the extracellular glycome, remains under explored. Recently, we have shown that CFT073 mutants deficient in the O-antigen chain of lipopolysaccharide and K2 capsule display growth-phase and cell density-dependent serum sensitivity. Additionally, it was shown that O-antigen and LPS outer core mutants displayed significantly reduced K2 capsule expression on the cell surface, coupled with increased unbound K2 capsule being detected in the supernatant relative to the wild-type. Free K2 capsule was shown to be protective against serum-mediated killing of CFT073. The R1 core was implicated in the tethering of K2 capsule to the CFT073 cell surface. In this study, fluorescence microscopy suggested CFT073 morphology is altered in LPS core mutants, which display punctate capsule expression.Moreover, the dependence of K2 capsule on the R1 core and O-antigen was further characterised. The protective effects of free K2 capsule were examined in a non-pathogenic K-12 background. Additionally, the ability of R1 core,O6 antigen and K2 capsule to confer serum resistance was examined in a K-12 background. Overall, these results highlight a crucial role for R1 core in CFT073 extracellular glycome expression as well as the complex regulation and interplay between lipopolysaccharide and capsule, a relationship which requires further characterisation.

B270

Plants Extract Harbouring Antimicrobial Activity Against the Acne-Causing Microbes S. Epidermidis

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Abstract

Skin is the first line of defence that form a complex barrier-like structure against the external environment, it harbours a commensal microbial community which, when disturbed might lead to infections and skin conditions such as acne. Acne vulgaris is an inflammatory disease that occurs in the sebaceous glands of the skin and is marked by the eruption of pimples. The disease is caused by opportunistic microorganisms such as *Propionibacterium acnes* and *Staphylococcus epidermidis* (Byrd, 2018 and Vora, 2018).

We tested *S. epidermidis* by agar well diffusion method against four plant extracts namely, *Azadirachta indica*, *Rhazya stricta*, *Camellia sinensis* and *Ocimum basilicum*. *S. epidermidis* growth was generally inhibited by all four compounds. The maximum antimicrobial activity was observed by acetone and methanolic extract of *A. indica*, *R. stricta*, *C. sinensis* and *O. basilicum* with mean ±SD of 30±1.1 and 28±1; 27±2.9 and 28±1.2; 25±1.4 and 27±1.8; 26±1.7 and 24±2.0 respectively. The minimum inhibitory concentration (MIC) of acetone and methanol extracts of plants were 25, 12.5mg/ml, 50, 25mg/ml, 100, 50mg/ml, 100,100mg/ml respectively. Here, we report a potential antimicrobial activity of these four plant extracts against the gram-positive bacteria *S. epidermidis.* Further work to pinpoint a common active compound would aid the development of a new antibiotics compound tackling the global antibiotics resistance conundrum.

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B271

An investigation into the mechanisms of bacterial adaptation in chronic Burkholderia cenocepacia cystic fibrosis lung infection

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Abstract

Chronic infection by opportunistic pathogens is the highest cause of mortality in people with cystic fibrosis (CF). Many bacteria adapt over time of colonisation to survive in the lung niche; however the adaptation mechanisms are not well understood. *Burkholderia cenocepacia* (Bcc) causes chronic, life-threatening infections in people with CF which are impossible to eradicate.

We previously identified a protein (BCAS0292) that is a potential regulator of the switch from acute to chronic infection in Bcc. A targeted deletion ΔBCAS0292 mutant showed the altered abundance of >1000 proteins and the loss of many phenotypic traits associated with persistence in the CF lung. Based on its structure we hypothesise that BCAS0292 is a DNA mimic protein, which functions by binding to DNA-binding proteins and modulating their activity.

The aim of this study was to identify the interactome of BCAS0292 and determine its mechanism of action. We performed an immunoprecipitation using FLAG-tagged BCAS0292 to elucidate its binding partners and identified three binding partners including two histone-like proteins, HctB and BCAM1012, and the protein translocase SecA. Identification of two histone-like proteins supports our hypothesis that BCAS0292 acts as a DNA mimic. The translocase activity of SecA and the localisation of HctB to the outer membrane may also explain the pleiotropic effects of BCAS0292 at the cell surface.

This study furthers our understanding of the mechanisms of bacterial adaption in chronic infection and will allow us to therapeutically target these mechanisms to prevent the switch to chronic infections in the lung.

B273

Novel T-cell based vaccines to fight ESKAPE infections globally

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Abstract

Antibiotic resistance is a major public health concern worldwide. Novel approaches are required to curtail the emergence of multi-drug antibiotic resistant pathogens. One appealing strategy is vaccination, which can reduce infection incidence, lower use of antibiotics and limit resistance spread. We hypothesise that T cell-mediated immunization is protective for bacterial pathogens such as *Acinetobacter baumannii, Klebsiella pneumoniae* and *Enterobacter cloacae* infections. These bacteria are members of the ESKAPE pathogens. To test our hypothesis we implemented a reverse vaccinology approach. Bioinformatic tools were used to construct the core pan-proteomes *of A. baumannii, K. pneumoniae* and *E. cloacae* using proteomes available on UniProt. Further, a reverse vaccinology pipeline was implemented to filter proteins based on their probability to be antigens, homology to human and mouse and size, followed by prediction of T cell epitopes. The core pan-proteomes of *A. baumannii, K. pneumoniae* and *E. cloacae* included 1318, 1931 and 2315 proteins, respectively. At the final stage of the pipeline 229, 346 and 444 putative antigens were identified for *A. baumannii, K. pneumoniae,* and *E. cloacae*, respectively. Further, *K. pneumoniae* and *E. cloacae* had 248 antigens in common, which have the potential to be cross-protective candidates. The antigens were subjected to molecular cloning prioritising proteins with the highest antigenic probability rank. Screening in a murine *in vivo* model allowed us to identify top antigenic candidates based on IFNγ responses and show production of antibodies against them. Further work is ongoing to test immunogenicity and protection in an *in vivo* model.

B274

Microbiome-derived antimicrobial peptides as therapeutic options for the treatment of *Acinetobacter baumannii* infections

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Abstract

Antimicrobial resistance is still a current and pressing global issue, especially in regards to the ESKAPE pathogens. One of the critical ESKAPE pathogens is *Acinetobacter baumannii*, which is gaining a reputation as a bacterial species able to evade and survive current clinical therapies. *A. baumannii* is an aerobic Gram-negative coccobacillus bacterium associated with bacteraemia, urinary tract infections and ventilator-associated pneumonia, and is typically viewed as an opportunistic pathogen.

Previous research has shown that clinical strains of *A. baumannii* have susceptibility to novel antimicrobial peptides, specifically those identified from a rumen/gut microbiome metagenomic dataset. This project explored the use of cationic antimicrobial peptides identified from a variety of gastrointestinal sources (the cattle rumen, the kudu faecal microbiome, and the chicken caeca) as potential therapeutic options for *A. baumannii*. The results showed that peptides from all 3 microbiomes were effective against a variety of sensitive and resistant *A. baumannii* strains, as well as some peptides having noticeable effects inhibiting biofilm production. The peptides were largely non-toxic when tested against human RBCs, and the majority of peptides tested demonstrated membrane destabilisation modes of action.

Further work is to be carried out to determine whether the peptides tested have a preference for certain cell membrane components, as well as analysis of the transcriptome to potentially identify the mechanisms deployed by *A. baumannii* in response to peptide exposure. With that in mind, these peptides, and antimicrobial peptides in general, are strong candidates for therapeutic use against the growing issue of antimicrobial resistant *A. baumannii*.

B275

Retrospective study of porcine circovirus type 2 (PCV2) genotypes in Northern Ireland from 1996-2006 and 2011-2015: Genotypic shift from PCV2a to PCV2b

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Abstract

Porcine circovirus type 2 (PCV2), is the causative agent of postweaning multisystemic wasting syndrome (PMWS) in pigs. PCV2 was first identified in 1998 and according to the most recent global analysis, nine PCV2 genotypes have been identified, namely, PCV2 a-i. To date, there has been no phylogenetic study published about circulating PCV2 strains in Northern Ireland. To address this issue and to explore the current situation, the open reading frame 2 (ORF2) of 22 PCV2 isolates dated 2011 - 2015 were analysed in the present study. Comparison of current strains to 28 archival strains dated 1997 - 2006 was performed. Eighteen percent of the current strains belong to PCV2a and 82% to PCV2b genotypes, compared to 36% of PCV2a and 64% of PCV2b for archived strains. PCV2c, PCV2d, PCV2e, PCV2f, PCV2g, PCV2h or PCV2i were not identified in the present study. These results indicate the ongoing genotype shift in the prevalence of PCV2a to PCV2b in Northern Ireland pig farms which occurred before the widespread vaccination against PCV2, whereas the recent genotype shift from PCV2b to PCV2d has occurred in other countries since the introduction of PCV2 vaccination. Given the observed high evolutionary substitution rate of PCV2, the emergence of new genotypes (PCV2d, PCV2e, PCV2f, PCV2g, PCV2h and PCV2i), which may be as a result of factors such as vaccination pressure and natural selection and emphasises the importance of continued monitoring of PCV2.

B276

Apparent re-emergence of Schmallenberg Virus in Northern Ireland

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Abstract

Schmallenberg virus (SBV) is an arthropod-borne virus Orthobunyavirus of the Simbu serogroup (family Bunyaviridae) which was first identified in Germany in 2011 and is transmitted by *Culicoides* biting midges. In adult animals, SBV causes diarrhoea, pyrexia, and a drop in milk yield. In pregnant females, SBV infection can lead to abortions, stillbirths and congenital malformations in new-borns.

SBV was first detected in Northern Ireland (NI) in stillborn ruminants in 2013 by real time RT-PCR. Subsequently, SBV was not detected until 2017 in an isolated case. Since the beginning of 2018 there has been a marked increase in positive cases of SBV in stillborn lambs tested by RT-PCR in NI. The overall sero-prevalence was 10.28% for cattle and 18.18% for sheep between January 2013 and July 2018. SBV was detected by PCR in 2.11% of bovine samples and 25.21% of ovine samples.

This study details an increased detection of SBV in NI with initial sequence analysis of a mutation hotspot being undertaken in selected cases. Comparative nucleotide analysis of the Hyper Variable Region fragments from selected cases (n=6), suggest NI SBV strains are ≥97.6% similar.

Phylogenetic analysis revealed that the NI SBV strains are highly similar to those identified in other parts of Europe exhibiting a phylogenetic relationship to SBV strains mainly from England and to a lesser extent from Germany and Sweden. Therefore, further analysis is required to determine if this represents a new incursion or is derived from strains detected in previous years.

B277

Hfq-CLASH reveals a novel mechanism of intrinsic β-lactam susceptibility in Klebsiella pneumoniae

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Abstract

Klebsiella pneumoniae (Kp) is a Gram-negative, multidrug resistant nosocomial pathogen that poses an urgent threat to human health. Kp utilizes post-transcriptional regulation mechanisms to rewire gene expressions levels using small non-coding RNAs (sRNA) and RNA binding proteins (RBPs) to adapt to stresses like antibiotic attacks. The functionality of various sRNAs is unknown. To understand the genetic mechanism involved, we performed CLASH – crosslinking, ligation, and sequencing of hybrids and unearthed a large number of novel sRNA-RNA interactions. Interestingly, we observed numerous chimeric interactions for an exponential phase sRNA, Spf. We characterized Spf interacting with the protein coding region of *mrdA* encoding PBP2 (Penicillin Binding Protein 2), which was previously uncharacterized in Gram-negative bacteria. Our QPCR expression analyses demonstrated a significant 0.8 fold decrease in *mrdA* levels in an Spf deletion mutant, which suggested the importance of Spf in stabilizing the mRNA. Cells lacking Spf exhibited 2-8 fold increase in resistance to β-lactam antibiotics, including ampicillin, carbenicillin, benzyl penicillin, mecillinam, and penicillin V. In conclusion, our work describes a novel mechanism of β-lactam susceptibility in Kp.

B278

Molecular genetics of the virulence plasmid of pathogenic *Escherichia coli* O104:H4

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Abstract

The exchange of mobile genetic elements has previously resulted in the emergence of atypical bacterial strains posing a huge risk to human health. One such example was the 2011 European outbreak of E. coli O104:H4 which carried an unusual combination of EAEC- and STEC-associated virulence factors on a plasmid and phage respectively. The E. coli O104:H4 virulence plasmid has exhibited unusual stability when subjected to a range of environmental stresses, in contrast to frequent plasmid loss in vivo. We have investigated the maintenance systems possibly linked to this unique stability focusing on the toxin-antitoxin (TA) systems involved in post segregational killing responsible for plasmid maintenance and therefore increased virulence. We have analysed the function of putative TA system genes present on the virulence plasmid by inducing their expression in lab made vectors in order to further understand the mechanisms involved in plasmid maintenance during infection. The disruption of these systems will then be assessed through the application of various environmental stresses resulting in plasmid loss and therefore the loss of key virulence factors from the cell. Following curing of the virulence plasmid via heat shock, we have further identified various phenotypic changes including reduced motility and increased aggregation in the plasmid-free variant. The 2011 European strain displayed heightened pathogenicity and provided unforeseen treatment challenges; our closely-related strain therefore provides a model for understanding MGE carriage to predict and combat future outbreaks of hybrid pathovars through the exchange of mobile genetic elements.

B279

Extracellular vesicles shaping the colorectal cancer microbiome

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Abstract

Colorectal cancer (CRC) is linked to a shift in the number of species in the gut microbiome. The underlying microbiota-gut interactions are not well understood yet. Extracellular vesicles (EVs) could be considered as mediators of gut-microbiome interactions. They mediate intercellular communications by delivering their cargoes to target cells. We hypothesize that regulatory cargoes within CRC-derived EVs may have an impact on gut microbiota through targeting the microbiome transcriptome. To assess the hypothesis, two CRC cell lines: SW480 and SW620 were cultured in cell culture bioreactors, EVs were isolated from the media of the cultures by size-exclusion chromatography and characterised by nanoparticle tracking analysis (NTA) and western blotting. The impact of the EVs on the bacterial phenotypic characteristics (growth curve, biofilm formation) was assessed. The uptake and interactions of florescent-labelled EVs by two different florescent-labelled strains of *E.coli*: MG1655 (Laboratory strain) and 11G5 (CRC-associated strain) was assessed by confocal microscopy. Through the NCBI Nucleotide BLAST, sequence complementarity between miRNAs and bacterial genome was identified. NTA showed a high number of particles with a characteristic EV size profile, CD63 and CD9 markers confirmed the presence of EVs. Preliminary data suggested that there is an impact of CRC-derived EVs on the bacterial phenotypic characteristics such as biofilm formation. Confocal images suggest an interaction between the EVs and *E. coli*. Potential targets for CRC-associated microRNAs across the *E.coli* transcriptome were identified by BLAST. Overall, revealing the host-driven microbiological regulation in CRC could facilitate the evolution of a new targeted treatment.

B280

Structures and Mechanisms in killing *C. difficile* by Bacteriophages

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Abstract

Clostridioides difficile is the leading cause of antibiotic-associated diarrhoea. Antibiotic exposure enables C. difficile to thrive by disrupting the protective gut microbiota and current treatments exacerbate this dysbiosis resulting in frequent recurrence. Therefore, new species-specific treatments are urgently needed. Bacteriophage-based therapies are a promising strategy for treating C. difficile, but exploitation will require a deeper understanding of the mode of action of these obligate intracellular parasites. For example, it is not known how phages recognise their cell surface receptors, the location of binding and what changes occur while undergoing binding and cell penetration. Therefore, understanding the binding mechanics of bacteriophages to the C. difficile cell-surface and how the subsequent cell envelope penetration occurs is critical to aid the design of new therapeutics. Consequently, our work focuses on understanding the 3D structural molecular detail of phage binding and penetration of the cell envelope. To achieve this we are determining the structure of selected C. difficile phage(s) using CryoEM and single particle analysis and combining this with electron cryotomography of phages bound to bacterial cells. By combining the gathered structural data we aim to develop a detailed molecular understanding of the infection process.

B281

The role of membrane lipid remodelling in the efficacy of phage therapy in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an important Gram-negative pathogen with intrinsic resistance to certain antibiotics. It is of particular significance in individuals with cystic fibrosis. Antimicrobial resistance (AMR) is a massive threat to global health, with a predicted 10 million people dying from resistant infections by 2050 if nothing is done to tackle it. A promising therapy for combatting AMR infections is phage therapy. However, more research needs to be done into the mechanisms which affect whether phage therapy is successful or not. One aspect that needs to be investigated is the role of the bacteria cell surface in phage therapy. Bacteria can undergo changes in the lipid makeup of their cell membranes in response to environmental factors. This has the potential to influence membrane proteins and lipopolysaccharides of Gram-negative bacteria, which are known phage receptors. The environments inside a human body will be very different to the *in vitro* environments often used to study phage therapy in the lab, therefore it is important to understand the effect this change in environment has on phage therapy. Our results indicate that the lipids that make up the outer membrane of *P. aeruginosa* can influence the efficacy of phage infection, although the mechanisms are yet to be shown. This could be a potential important factor to consider in the development of phage therapy.

B282

Development and infection of bovine gut models with different strains of *Mycobacterium avium ssp paratuberculosis* to physiologically represent a natural infection

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Abstract

Mycobacterium avium paratuberculosis (MAP) is the etiologic agent of Johne's Disease (JD), a chronic gastric enteritis of ruminants world-wide hypothesised to be linked to Crohn's disease in humans. The current control methods for JD are ineffective and do not reduce spreading of the disease or infection. Better diagnostic tools and vaccine targets must be identified if we hope to mitigate the impact of JD on the economy and improve animal welfare. To identify new diagnostic and therapeutic targets specific to MAP the early interaction between MAP and the host must be investigated. Due to the slow-growing nature of MAP studying infection *in vivo* is difficult. The recent development of bovine enteroids has allowed the study of MAP in an *in vitro* model which is reproducible and physiologically relevant. We have characterised the cell-types present in 3D bovine enteroids, newly established 2D monolayers and 3D inside-out enteroids and compared them to bovine intestinal tissue using RT-qPCR and immunofluorescence staining to assess how representative the organoids are of the bovine intestine. The invasion efficiency and host cell targeting of MAP was assessed using two strains: the K10 reference strain, hypothesised to be lab-adapted, and a recent sequenced field isolate, MAP strain C49. Differences between strains may highlight the limitations of the widely used K10 in its ability to represent a typical MAP infection. These studies will highlight key mechanisms by which virulence MAP enters the host to establish infection and will impact the identification of diagnostic and vaccine targets for MAP.

B283

The effect of manuka honey on *Burkholderia cenocepacia* viability, antibiotic susceptibility and biofilm formation.

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Abstract

Cystic Fibrosis is a common genetic disorder, the hallmark of which is increased mucous viscosity, allowing colonisation of the lungs by opportunistic pathogens. Repeated rounds of antimicrobial treatments and presence of biofilms means that pathogens become highly resistant to antibiotics. Inflammation in response to the presence of bacteria drives irreversible lung damage and decreased lung function, ultimately only able to be resolved by transplant. The presence of some bacterial species, including *Burkholderia cenocepacia* complex (BCC), is associated with poor post-transplant prognosis. BCC is inherently antimicrobial resistant so novel treatment strategies are required.

The antimicrobial activity of manuka honey was previously demonstrated, alongside synergistic action with several antibiotics. Here, we combined manuka honey with a range of antibiotics commonly used to treat BCC and assessed activity against a panel of clinical BCC isolates. Although most isolates showed antibiotic resistance, all the strains tested wee susceptible to was <10% w/v manuka honey. Many isolates also displayed synergy when sub-MIC concentrations of honey and antibiotic were combined. Biofilm formation was similarly affected, with both reduced viability and biomass after 24 hr of treatment.

This work highlights that isolates of *B. cenocepacia* are susceptible to manuka honey. Further to this, we have demonstrated its ability to improve the activity of clinically relevant antibiotics. With further *in vivo* investigation there is potential for novel formulations to be developed. Eradication of *B. cenocepacia* from patients has important clinical ramifications and could allow previously ineligible patients to receive a lung transplant.

B284

Characterising the cellular tropism of Streptococcus pneumoniae with tissue resident macrophages following ex vivo infection of the human spleen.

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Abstract

Splenic tissue resident macrophages constitute a major role as one of the first lines of innate cellular defence against systemic infection. Unfortunately, whilst well characterised in location and cell-surface protein expression, there is a paucity of information regarding the functionality of this diverse set of human splenic tissue resident macrophages, with most functions inferred from murine models.

We have developed a clinical trial (ClinicalTrials NCT04620824) to collect human spleens for *ex vivo* perfusion and infection. Organs are perfused for up to 6 hours utilising synthetic oxygen carriers and are infected with $1x10^7$ CFU of single strain or multi-serotype Streptococcus pneumoniae doses.

Confocal and scanning microscopy data show that bacterial numbers rapidly increase in the spleen, while decreasing in the perfusate. Over time, the number of intracellular bacteria in macrophages decreases. When considering the macrophage subpopulations, the CD68+CD163+CD169- red pulp macrophages are associated with the largest percentage of bacteria. Once normalised, data show that both the perifollicular CD68+CD163-CD169+ and non-perifollicular CD68+CD163-CD169- capillary sheath-associated macrophages are more likely to contain bacteria in some instances. Co-infection with type 2 and type 4 serotype strains does not strongly indicate significant differences between the strains in cellular tropism.

This is the first human-specific evidence demonstrating differential pneumococcal association with multiple subpopulations of splenic tissue macrophages in the first hours following infection, underlining the notion that tissue resident macrophages are heterogeneous in their role in response to invasive infection, alongside also highlighting the importance of considering antimicrobials with high intracellular activity as treatment options.

B285

Dissecting *Klebsiella pneumoniae* survival in human endothelial cells, a new host-pathogen battleground

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Abstract

Klebsiella pneumoniae causes pneumonia and disseminated infections that lead to sepsis in humans. Sepsis is a rapidly progressing disease where the window for treatment is short and where mortality for multidrug resistant *K. pneumoniae* sepsis is 42%. Microvascular endothelial cells are key regulators of the peripheral vascular system in infection and inflammation and are often the first contact against invading bacteria. However, the interaction between these cells and sepsis-triggering pathogens has been largely overlooked. This study aims to dissect the interaction between *K. pneumoniae* and microvascular endothelial cells. The capsule polysaccharide limits the engulfment of *Klebsiella* by endothelial cells, and we found a correlation between the levels of capsule expressed by *Klebsiella* and the number of engulfed bacteria. However, intracellular bacteria survive over time showing the capsule is not necessary for survival. We obtained similar results testing *Klebsiella* hypervirulent strains and those of the global epidemic clone ST258. The cell viability of the cells was not affected by *Klebsiella* infection. Interestingly, live bacteria egress from cells, reminiscent of the phenotype reported for the urinary epithelium. We have established an *ex-vivo* model using rat mesenteric microvessels, recapitulating the infection results obtained probing the 2D cell model. This ex vivo model allows the possibility to determine physiological changes in the vessel during an infection with *K. pneumoniae* and in the presence of vasoactive drugs. Altogether, this work describes a hitherto unknown host-pathogen interaction relevant to the pathology of sepsis.

B286

Comparison of circulating molecular types of *Bordetella pertussis* in England, from cultures and directly from clinical specimens

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Abstract

Background and Aims

Whooping cough is caused by *Bordetella pertussis*, an important cause of morbidity and mortality, particularly in infants. The UK Health Security Agency National Reference Laboratory (NRL) requests submission of *B. pertussis* isolates for surveillance. From 01/2015, primary PCR testing was offered by the Specialist Microbiology Services Laboratories and PCR positive samples referred to the NRL for further characterisation.

We reviewed submissions to the NRL from 01/2015 to 03/2019.

Methods

Isolates (n=351) and clinical specimens (n=484) were analysed using existing schemes for multilocus-variable-number-tandem-repeat-analysis (MLVA) and DNA typing of pertactin, pertussis toxin (PT), and PT promoter genes (*prn*, *ptxA*, *ptxP*).

Results

Age range/median age of patients from which isolates and clinical specimens were taken was <1y-81y/<1y and <1y-86y/<1y respectively.

Complete MLVA profiles were obtained from 349/351 (99.4%) isolates and 210/484 (43.4%) clinical specimens. The predominant MLVA type was 27 in both isolates 275/349 (78.8%) and clinical specimens 154/210 (73.3%) with complete profiles respectively. Complete sequence typing results were obtained from 342/351 (97.4%) isolates and 102/484 (21.1%) clinical specimens. The dominant combined profile of *prn2*, *ptxA1*, *ptxP3*, MLVA 27 accounted for 267/340 (78.5%) of isolates and 62/78 (79.5%) of clinical specimens with a full sequence and MLVA profile.

Conclusions

Increase in pertussis PCR testing has led to a decline in referred isolates. Molecular epidemiological studies can be achieved without culture, but low target DNA yield affects the success of such analyses. The dominant profile shown here arose more than 10y ago and has remained highly dominant.

B287

Characterisation of capsular serotypes of *Streptococcus pneumoniae* and its role in virulence following infection of spleen and liver tissue macrophages

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Abstract

Invasive pneumococcal infections remain as one of the highest cause of morbidity and mortality in young children, despite vaccination programs. The spleen and the liver have an intricate network of innate immune cells against blood borne pathogens. Unfortunately, characterisation of bacterial and tissue resident macrophage dynamics within these organs remains to be elucidated. An experimental murine septicaemia model was used to analyse the differences between pneumococcal strains and its virulence. A significant increase in blood counts was observed in “mouse virulent” (BS71, BHN418, BHN191, D39 and TIGR4) pneumococci strains compared to “non-mouse virulent” (BS69, BHN100, CBR206, LgST215, DP1004 and G54) strains, in which clearance from the blood was shown within 24 h of infection. Authorisation was obtained for the perfusion and infection of human spleens and livers (ClinicalTrials gov NCT04620824 REC 18/EM/0057 and REC: 21/PR/0287) to examine potential discrepancy between mice and human models as well as differences in pneumococci virulence. The organs were infected with 10^7 CFU for up to 6 h with multiple pneumococci strains and serotypes. Data from confocal and scanning microscopy revealed an initial increase in bacterial numbers in the spleen and rapid decline over the course of infection. Specific subtypes of macrophages in the spleen were shown to have preferential association with intracellular pneumococci in comparison to others. This highlights the importance of splenic macrophages in bacterial clearance during the initial stages of infection and the value of effective antimicrobials with intracellular activity.

B288

Development of a preclinical framework for use in formulation of antimicrobial therapeutics against cystic fibrosis infection (PIPE-CF)

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Abstract

Chronic bacterial infection poses a significant burden on people with cystic fibrosis (PwCF), increasing morbidity and mortality associated with the disorder. PwCF are prescribed a range of antimicrobial therapeutics, which can in themselves become burdensome. There is a pressing need for the development of novel antimicrobials to lower this burden and combat rising antimicrobial resistance. Current models used in preclinical testing poorly mimic the respiratory environment, resulting in low translation between preclinical and clinical endpoints. This has hindered progress, as the preclinical efficacy of antimicrobial compounds is often unrepresentative of performance in patients.

This project aims to develop an evidence-based, standardised antimicrobial development pipeline for CF therapeutics (PIPE-CF). Each stage of development will be evaluated, including the selection of a bacterial strain panel for screening; development of polymicrobial biofilm screening tools; investigation into the influence of host-pathogen interactions; validation of existing preclinical models; integration of relevant pharmacokinetic and pharmacodynamic models; consideration of adaptability for different CF pathogens; and the creation of online resources to assist the design of preclinical screening projects. Here, initial strain choice and baseline data will be presented, alongside a framework for future testing.

The pipeline should accelerate drug discovery, increasing the consensus between preclinical and clinical outcomes. PIPE-CF will bring together academics, clinicians, industry, regulators and PwCF. Its development will improve the reliability of preclinical testing, increasing confidence and investment in the sector. PIPE-CF will substantially and continuously benefit PwCF, as it is continuously utilised and optimised in the future.

On behalf of the PIPE-CF consortium.

B290

Defining the role of efflux in bacterial biofilm formation and antimicrobial resistance to develop new treatments for infection

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Abstract

Catheter associated urinary tract infections (CAUTIs) are a common healthcare associated infection and present a significant burden to health services. The urease producing pathogen Proteus mirabilis (*P. mirabilis*) is a common causative agent of CAUTIs due to crystalline biofilm formation on the catheter surface leading to blockage. Biocides such as chlorhexidine (CHD) are a common component of products used to flush through catheters and reduce biofilm and CAUTI development, however many clinical *P. mirabilis* isolates show high tolerance to CHD. This tolerance is also associated with resistance to other antimicrobials. Both biofilm formation and CHD tolerance have been linked to efflux pump activity in *P. mirabilis*, therefore the development of efflux inhibitors could be a valuable approach to aid the treatment of CAUTIs. Drug repurposing has the potential to decrease the development time for these desperately needed treatments. Key efflux systems with involvement in biofilm formation and resistance have been identified by mapping phenotypic characteristics to variants identified within efflux related genes in a clinical isolate panel, alongside expression data obtained in clinically relevant conditions. Ongoing work is analysing the effects of the knockout of these systems on biofilm formation in the *in vitro* bladder model, and on virulence *in vivo* using the *Maduca sexta* model. An *in silico* model will be then used to identify candidate inhibitors of the identified systems within existing compound databases. The effects of chemical inhibition will be analysed with the hope of identifying potential candidates for repurposing as efflux inhibitors.

B291

Probiotic and pathogenic biofilm-associated bacteria drives immunoregulatory response in host innate and adaptive immunity

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Abstract

Introduction: The gastrointestinal tract is colonized with commensal microorganisms, predominately comprising of bacteria. Disruption of the gut microbiome favours bacterial infection and inflammatory diseases (e.g., inflammatory bowel disease). Probiotic bacteria can decrease pro-inflammatory responses and induce differentiation of regulatory T cells that suppress immune responses, hence limiting hyper-inflammatory reactions. However, the precise mechanisms behind such effect remain unclear. Macrophages (MΦ) are phagocytes that engulf infectious agents and activate naïve T-cells through presenting pathogen-derived antigens. We hypothesize that (i) bacterial subunits (including biofilms) impact on MΦ differentiation and the consequent T cell response; and (ii) probiotic rather than pathogenic bacteria-derived subunits favour immunosuppression.

Methods: Peritoneal MΦ and spleen lymphocytes were extracted from Balb/c mice and cultured (2 and 6 days, respectively) with subunits (whole dead bacteria and heat-killed biofilms) derived from different bacteria: *Lactobacillus casei* strain Shirota, *Lactobacillus reuteri*, *Enterococcus faecalis*, *Acinetobacter baumannii* and *Staphylococcus aureus*. Thereafter, immune cell activation was assessed by flow cytometry.

Results: After 2 days, compared to whole dead bacteria, F4/80+ MHC II low MΦ stimulated with *L. casei* Shirota and *S. aureus* biofilm showed an increased expression of the immunoregulatory CD200R with lower inflammatory cytokine, TNF-α. In parallel, higher activation of CD4+ T cells was observed post 6 days culture of whole dead bacteria than heat-killed biofilm.

Conclusion: Compared to dead *S. aureus* cells, *S. aureus* biofilms have anti-inflammatory effects on peritoneal macrophages via reducing pro-inflammatory cytokine production and increasing immunoregulatory receptors.

B292

Improving Intracellular Delivery Of Bacteriophages Using Liposome-Based Encapsulation

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Abstract

Due to the continuing spread of antimicrobial resistance, phage therapy has regained interest as an alternative treatment option for bacterial infections. In recent years, the research on phage therapy has achieved substantial progress, but its clinical application still has many difficulties, including the narrow specificity range of bacteriophages and the rapid elimination of phages by the host immune system. In addition, many important human bacterial pathogens are intracellular (e.g. *Salmonella* spp.), making it more difficult for phages to reach them through the cellular membrane. This presents a problem for phage therapy against such pathogens, reducing the efficiency of phage delivery and subsequent bacterial lysis. In this project, we aim to develop a liposome formulation to improve the viability and stability of therapeutic phages *in vitro* and *in vivo*. The phospholipid bilayer of liposomes readily fuses with cell membranes, making them one of the preferred methods of delivery of small molecules and biologicals, so liposomes can penetrate cell membranes to transport phage into cells and increase the retention time of phage particles at the site of infection, which presents a promising strategy for the treatment of infections with intracellular pathogens. Herein, we have selected several broad host spectrum phages (e.g. *Salmonella* phage Felix O1) and loaded them into liposomes. The morphology, size, encapsulation efficiency and stability of the resulting phage-loaded liposomes were extensively characterised. Preliminary experiments have demonstrated encouraging results and we expect that the encapsulated phage cocktails would improve the efficacy of treatment in the *in vivo* models of infection.

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KiVA - Identification of *Klebsiella pneumoniae* Vaccine Antigens.

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Abstract

Klebsiella pneumoniae is a gram-negative bacterium, which causes respiratory, urinary tract and wound infections. Pneumonias caused by K. pneumoniae are difficult to treat and mortality rates can reach up to 50% even after antibiotic therapy. The WHO reported that 80% of Klebsiella pneumoniae infections were resistant to ciprofloxacin, therefore there is an urgent need to develop an efficacious vaccine.

The aim of this project is to identify proteins used by K. pneumoniae to attach to host epithelial cells, with a view to identifying novel protective vaccine antigens. We measured the ability of two K. pneumoniae strains, CIP52.145 and capsule mutant, CIP52.145△cps to attach to lung epithelial cells, A549 and 16HBE14o-. The CIP52.145 strain showed 7.3-fold higher binding to 16HBE14o- cells relative to the CIP52.145△cps strain, while the latter showed 4.7-fold higher binding to A549 cells. Subsequently, we used a proteomic technology approach to identify novel bacterial adhesins. In total, 31 bacterial proteins were common to both strains involved in attachment to both epithelial cell lines, of which, 23 were never previously identified as potential vaccine antigens of K. pneumoniae. The proteins were short-listed based on novelty, breadth across K. pneumoniae strains and absence or lack of conservation in E. coli strains. Finally two were cloned into E. coli BL21 (DE3), expressed and purified and their role in attachment confirmed. E. coli BL21(DE3) expressing Antigen L showed 13.4-fold increased attachment, while E. coli BL21(DE3) expressing Antigen D showed 7.2-fold increase. These novel antigens will be examined for their vaccine potential.

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Early onset meningeal pathogenesis with K1 Escherichia coli confers major foci formation in the vascular areas of the brain in CD1 mice

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Abstract

Escherichia coli is a major human commensal and a leading pathogen when it comes to a plethora of diseases. The K1 capsule polysaccharide subclass of E. coli is mostly associated with neonatal meningitis and adult sepsis. However, it is prevalent in cases of UTI and has been found to colonise the human gut without any signs of disease. Following an intravenous infection in adult CD1 mice, E. coli successfully reached the brain and established itself in the vascular zone of the meninges within the first 12h. We observed that past this point mice became septic, preventing successful following of disease progression. To monitor E. coli pathogenesis in the brain, mice were treated with 40 mg/kg cefazolin at 12h and 24h post infection. Over a period of 72h, the bacterial presence in the blood gradually dropped below detection limits, whilst CFU could be recovered from the brain at elevated levels throughout. Confocal microscopy analysis at 24h post infection showed a minute presence of E. coli. Most observed bacteria were located within the region of the vascular endothelium (CD31+). At 36h bacterial signal was associated with CD169+ perivascular macrophage; the majority of E. coli seen as single cells. At 48h the bacterial signal is located at vessel rich areas (i.e. meningeal, choroid plexus) with large foci (bacterial n >20) detected throughout. The foci numbers reduced over the next 24h, suggesting a potential tendency towards recovery. These results imply a major replication event in the vascular areas of the brain following the early onset of K1 E. coli driven meningitis.

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