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POSTER ABSTRACT BOOK

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01

Extracellular vesicles shaping the colorectal cancer microbiome

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Abstract

Colorectal cancer (CRC) is a complex disease which is linked to gut dysbiosis. Although the causative link between CRC and microbiota is widely investigated, the underlying microbiota-gut interactions are not well understood yet. Extracellular vesicles (EVs) could be considered as mediators of gut-microbiome interactions. They are cell-derived membranous vesicles that carry various types of cargos such as proteins, DNA, and RNA. They mediate intercellular communications by delivering soluble mediators and effectors 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 NanoFCM analysis and western blotting. 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. NanoFCM data showed a high number of particles with characteristic EV size profile, and CD63 and CD9 markers confirmed the presence of EVs. 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. Overall, revealing the host-driven microbiological regulation in CRC cancer could significantly facilitate the evolution of a new targeted treatment strategy.

P02

Engineering bacterial photonic crystals: Novel Colour Generation

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Abstract

Structural colour (SC) is a consequence of light interacting with ordered nanostructures, and it is responsible for the most vivid and brightest colours in nature. Despite there is a plethora of work describing the frequency and diversity of SC in living organisms, little is known on the underlying genes, biochemical pathways, and evolution at the basis of SC. To approach this problem, we use as model organism, *Flavobacterium* IR1, which rapidly self-organises into an ordered nano-scale 2D periodic lattice crystalline colony, angle-dependent coloured patterning when illuminated with white light. This assembly process offers the potential to create engineered living materials with aesthetic and functional properties.

IR1 is genetically amenable, allowing exploration of the genes that specify colony organization and to create SC. We first generated a transposon insertion mutant library and conducted comparative proteomic analyses on selected IR1 SC mutants. This work has been performed to identify biologically relevant proteins and pathways involved in SC. Furthermore, a novel CRISPR-Cas gene-editing tool has been adapted for IR1, able to knockout genes to elucidate the SC formation and to create new enhanced bacterial photonic properties. We aim to develop new bacterial coloured optical structures, as potential substitutions for unsustainable pigments and as biosensors

P03

Polymer-Based Inhibition of Quorum Sensing in Gram-Negative Bacteria

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Abstract

Quorum sensing (QS) is a form of cell-to-cell communication that regulates several bacterial virulence phenotypes. One form of QS signalling in Gram-negative bacteria involves the signalling molecule acyl-homoserine lactone (AHL), which acts as an autoinducer to stimulate the expression of several virulence factors. An anti-quorum sensing polymer, HB-PNIPAM-HL, has recently been developed by our group. HB-PNIPAM-HL is a highly branched Poly (NIPAM) polymer with homoserine lactone ends that is suggested to interfere with the AHLs QS system. Targeting QS circuits could present a possible control over several virulence factors at once and thus limit infection. This project investigates the extent of the circuit disruption via HB-PNIPAM-HL in a biosensor strain of Gram-negative bacteria, *Chromobacterium violaceum* CV026, and the Gram-negative pathogen *Pseudomonas aeruginosa* PAO1. The anti-QS ability of the polymer was first measured in the *cviI* mutant strain of *Chromobacterium violaceum* CV026. Gene expression of receptors involved in AHL-type signalling in the presence or absence of HB-PNIPAM-HL was investigated using RT-QPCR. The effect of the polymer on *Pseudomonas aeruginosa* PAO1 virulence factors regulated by QS was then evaluated via several virulence factors assays. The polymer was also investigated in a 3D skin model at two treatment periods, after 2 and 24 hours of infection with *P.aeruginosa*.Initial tests using the CV026 biosensor assay indicated HB-PNIPAM-HL was able to Interfere with QS signalling and downregulated some *P.aeruginosa* virulence-related phenotypes that QS controls. The initial data suggest that HB-PNIPAM-HL can interfere with QS signalling.

P04

Necrotising fulminant mucormycosis in an operated open femur fracture – a survivor's tale

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Abstract

Introduction: Life-threatening mucormycosis has rarely been reported. Mortality rates of 50-100% make the earliest recognition of this highly fulminant infection, critical.

Case Discussion: A 23-year-old male was referred from a semi-urban centre with 15-days of fever and a non-healing left thigh wound. He sustained a Gustilo-Anderson-IIIA open femur shaft fracture from a two-wheeler collision with a tree. Femoral nailing had been performed at the referring centre 2 days post-injury. His open wound kept discharging pus and he was referred for further management.

Deeper questioning from parents revealed the patient's history of consuming weight-gain dietary supplements at his local gymnasium. His left thigh was grossly swollen and tender with a large 30x40cm black-grey, necrotic mass with powdery scales exposing underlying muscles. Repeated swabs revealed no significant growths and he deteriorated rapidly despite broad-spectrum antibiotics. Urgent multidisciplinary (orthopaedic-medicine-microbiology) discussions resulted in microbiologist-led sampling from the lesion's edge. This revealed broad, branching, irregular and septae tangles of fungal hyphae of mucormycosis.

Liposomal Amphotericin-B therapy was immediately initiated and maintained (5mg/kg/day) for 2 weeks. Repeated aggressive surgical debridements complemented this. The patient’s condition improved dramatically. A large split-skin graft was then done over granulating tissue. After 6 weeks of stay, the patient went home walking. Serial follow-ups continued to show excellent improvements with good graft-take at 2 years.

Conclusion: Mucormycosis presents as a rare, life-threatening infection. Clinical suspicion is warranted in potentially immunosuppressed patients and contaminated wounds. Specific medical and radical surgical treatment can make the difference between life and death.

P05

Pathogenesis of otitis media: the role of epithelial cells' PAFr and NTHi's PCho in the invasion of middle ear epithelium

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Abstract

Otitis media (OM) is an infection of the middle ear (ME) and one of the most prevalent diseases in young children. It can lead to hearing loss, cholesteatoma, and meningitis and represents a substantial financial burden as it is a main cause of pediatric antibiotic prescription and surgery; the rapidly rising numbers of AMR bacteria, however, mean alternative therapies are urgently needed.

NTHi, one of the most common otopathogens in acute OM, is an opportunistic pathogen of the human respiratory tract, commonly associated with respiratory infections. Interactions with airway epithelial cells have been investigated, however little is known about its contribution to disease in the ME. Interactions of NTHi with cell receptors, such as platelet-activating factor receptor (PAFr), through lipooligosaccharide (LOS) phosphorylcholine (PCho), have been linked to the invasion of airway epithelial cells. Still, to date, only the adhesion of NTHi in ME has been investigated.

This study investigates the mechanisms of NTHi infection and invasion by examining the effects of NTHi PCho on human ME epithelial cell invasion using gentamicin protection assays. Invasion of strains differentially expressing the protein, H457 (wt), H446 (PCho-), H491 (PCho+) will be assessed. The role of epithelial PAFr in the invasion of bacteria will also be determined by blocking bacterial access to the receptor via competitive binding with a PAFr antagonist, ABT-491. A better understanding of how NTHi evades the immune system and promotes persistence will allow us to elucidate novel therapeutic strategies, avoiding repetitive use of antibiotics.

P06

Comparative analysis of Myxococcus and Streptomyces genomes and predatory activities

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Abstract

The phyla Actinobacteria (now Actinomycetota) and Myxobacteria (now Myxococcota) both contain important soil-dwelling organisms. Despite Actinomycetota being mostly Gram-positive and Myxococcota Gram-negative, they contain genera (Streptomyces and Myxococcus respectively), which have very similar biological properties. Both genera have huge genomes (often exceeding 10 Mb, with a high GC content), are predators of other bacteria, produce antibiotics, and have complex lifecycles involving differentiation into spores. In this paper, we conducted a comparative analysis of the genome sequences and the predatory activities of Streptomyces and Myxococcus strains (including novel isolates), with the objective of further categorising their antibiotic production. Soil dwelling bacteria were isolated from woodland using standard microbiology techniques. 16S rRNA gene sequencing confirmed the isolation of a novel Streptomyces sp.(strain C4A), with sequence identity to the nearest Streptomyces type strain (Streptomyces albidoflavus) of just 88.73%. Draft genome sequences were obtained and annotated using the prokaryotic genome annotation pipeline, Prokka. Pan-genomic analysis revealed a surprising degree of openness of the Streptomyces spp. pan-genome, and is different to other Actinomycetota, but similar to that of Myxococcus spp. The number of metabolites across 3 Streptomyces isolates are 6, 26 and 64, which came as an output using anti-SMASH. On corelating the openness of the pan-genome and predatory profiles of Streptomyces spp. isolates which were assayed by testing predatory activity against a range of plant pathogens, the results demonstrate a marked difference in predatory profile compared to those of Myxococcus xanthus, thereby making it any interesting avenue to explore for tackling antimicrobial resistance.

P07

Realising the *POT*ential of POT transporters; deciphering the interactions between bacterial peptide transporters and antibiotics

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Abstract

The prevalence of antimicrobial resistance in bacterial pathogens has risen exponentially in recent decades as a result of clinical overreliance on these compounds. There is now a growing need to identify the intrinsic physiological factors which contribute toward antibiotic efficacy, in order to better understand how bacteria overcome antibiotic therapy and to inform the design of novel therapeutics.

Proton-dependant oligopeptide transporters (POTs) facilitate the uptake of di- and tri-peptides as a nutrient source across a wide range of bacterial species. Emerging evidence suggests that bacterial POTs may provide a route of uptake for various peptidomimetic antibiotics into bacterial cells, including clinically relevant β-lactams such as amoxicillin. Here, we aim to further characterise the interactions between bacterial POTs and antibiotics and assess the impact of these transporters on drug susceptibility. To this end, we have generated E. coli and S. aureus mutants which lack their native POT transporters. Ongoing bacterial growth assays in the presence of antibiotic consistently demonstrate an increased β-lactam susceptibility phenotype in POT mutant strains when compared to wild-type, suggesting a previously unappreciated role for these transporters in intrinsic β-lactam resistance. Conversely, a POT mutant strain of S. aureus shows decreased susceptibility to inhibitory concentrations of the fluoroquinolone antibiotic ciprofloxacin, suggesting these transporters may provide a route of uptake for the drug. Future work will utilise a combination of further antibiotic susceptibility experiments alongside in vitro transporter affinity assays to more comprehensively elucidate the importance of POTs in antibiotic-mediated antimicrobial therapy.

P08

Stopping a killer superbug: unravelling the molecular mechanisms of *C. difficile* sporulation

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Abstract

Clostridioides difficile is an antibiotic resistant bacterial human pathogen that colonises the large intestine in the absence of a healthy competing gut microbiota. Current treatments for *C. difficile* infection (CDI), the most common cause of healthcare-associated gastrointestinal disease, entail use of antibiotics that perpetuate gut dysbiosis and enable disease recurrence. This highlights an urgent requirement to further understand the fundamental biology of this pathogen so novel therapies can be sought. A promising, albeit unexploited, target for new therapeutics is the process by which *C. difficile* forms spores, dormant cell forms resistant to environmental stressors and the primary CDI infectious agent. Sporulation begins with an asymmetrical cell division, and entails substantial peptidoglycan remodelling as the smaller forespore is engulfed by the larger mother cell prior to spore maturation. The molecular details of sporulation require further unravelling to find potential pharmaceutical targets to interrupt this process, thereby eliminating the CDI transmission route. SpoIIP is a dual amidase and endopeptidase involved in peptidoglycan remodelling during engulfment, but the localisation of SpoIIP *in vivo* and the identity of its endopeptidase catalytic residues remain unknown. Previous work has identified two cleavage sites targeted by the SpoIVB2 peptidase, proposed to be a mechanism of liberating enzymatically active SpoIIP isoforms. Our current work seeks to further investigate this, with each isoform having been generated using inverse PCR and tested for enzymatic activity using peptidoglycan digestion assays. By furthering understanding of the mechanisms of forespore engulfment, the crucial pursuit of novel CDI therapies will be aided.

P09

How the mobility of the transposon *Sanctuary I* influences the expression of the virulence gene *ToxA*

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Abstract

Horizontal gene transfer (HGT) is the movement of genetic material from two unrelated species, this mechanism leads to rapid genomic evolution by increasing the ability to acquire beneficial genes to occupy a new niche. A transposon-mediated HGT event of the virulence gene *ToxA* was discovered in three fungal wheat pathogens: *Bipolaris sorokiniana*, *Pyrenophora tritici-repentis* and *Parastagonospora nodorum*. *ToxA* is a necrotrophic effector and acts in an inverse gene-gene interactions, its function is dependent on the presence of the wheat disease-resistant gene *Tsn1*. *ToxA* was discovered to be enclosed in a class II DNA transposon named *ToxhAT*. In *B. sorokiniana*, *ToxhAT* was also seen to reside as a passenger in a larger ~200kb class II DNA transposon, named *Sanctuary I*. Through genome sequencing of eight Australian *B. sorokiniana* isolates, both *ToxhAT* and *Sanctuary I* were shown to be active and mobile. In each genome, *Sanctuary I* was found in a unique chromosomal location, thus *ToxhAT* was also found in a unique location, unlike the other two fungal pathogens. Each isolate could be further grouped by the genomic location of *Sanctuary I* on each chromosome and configuration of *Sanctuary I*. To investigate the effect that genome location has on the expression of *ToxA*, we conducted quantitative PCR of both *in vitro* and *in planta* growth. Here we observed *ToxA* is only induced *in planta*, regardless of the presence of *Tsn1*. We also see a clear tread in difference of *ToxA* expression between isolates carrying *Sanctuary I* on different chromosomes.

P10

Optimising a method for investigating the bovine respiratory syncytial virus immunopeptidome by mass spectrometry

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Abstract

The development of safe and effective vaccines against bovine respiratory syncytial virus (bRSV) continues to remain a high priority. Peptide viral epitopes that can trigger a primary and memory immune response have the potential to inform vaccine design to improve efficiency and reliability. This requires identification of viral peptides displayed by the major histocompatibility complex class I (MHC-I) that are recognised by CD8+ T cells. The MHC-I region of cattle is highly polymorphic and varies in gene content between haplotypes. Understanding the contribution of each cattle MHC-I allele to the total immunopeptidome is important if we are to identify immunogenic peptides presented consistently across cattle haplotypes.

To better understand the bovine immunopeptidome, we have optimised an immunoprecipitation method to capture MHC-I molecules from lysed cells. Uninfected and bRSV-infected Madin-Darby Bovine Kidney (MDBK) cells will be immunoprecipitated and the MHC-I bound peptides purified by High-performance liquid chromatography (HPLC). Analysis by Liquid chromatography tandem mass spectrometry (LC-MS/MS) will determine the sequences of the presented peptides and permit the identification of immunogenic peptides that may be of importance in bRSV immune detection. Similarly, the same protocol will be followed with MHC-I-null 721.221 cells transfected with individual bovine MHC-I alleles, to address each allele’s contribution to bRSV detection.

bRSV peptides identified in this study would be of interest in the design of peptide vaccines as we can be confident these pathogen peptides reach the cell surface during infection. The methodology represents a useful tool for better understanding the host-virus immunopeptidome in cattle.

P11

Using MGN-3 to mediate innate immunity in a diabetic (hyperglycaemic) model of an infected chronic wound.

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Abstract

Background

The diabetic foot ulcer (DFU) frequently becomes infected by polymicrobial communities, leading to patient morbidity and mortality. Although antibiotics are the first line of defence against DFU infection, over-usage has led to widespread antibiotic resistance. Given the need for novel therapies to replace or use alongside antibiotic intervention, this study investigated Biobran/MGN-3 as a potential modulator of innate host responses to wound pathogens in a diabetic (hyperglycaemic) model of an infected DFU.

Methods

Host-pathogen interaction assays (n=12) were used to assess the effect of MGN-3 on M1 (classically activated macrophage)-mediated phagocytosis of Gram-positive methicillin-resistant Staphylococcus aureus (MRSA) and Gram-negative Pseudomonas aeruginosa (PA01) under euglycemic (11mM) and hyperglycaemic (15mM, 20mM and 30mM) conditions. The phagocytic ability of M1 exposed to MGN-3 (0.5, 1.0, 2.0 mg/ml) was compared against bacterial clearance in the absence of MGN-3 (untreated control) or following treatment with either rice starch (2.0 mg/ml; negative control) or bacterial lipopolysaccharide (LPS 5mg/ml; positive control).

Results

Increasing levels of hyperglycaemia significantly ($p<0.05$) increased bacterial recovery by impairing M1-mediated phagocytosis. However, MGN-3 and LPS supplementation reversed the detrimental effect of glucose by significantly increasing ($p<0.05$) phagocytosis of both MRSA and PAO1 in a dose dependent manner compared to untreated and negative controls.

Conclusion

MGN-3 significantly reversed the detrimental impact of increasing hyperglycaemia on M1-mediated phagocytosis, highlighting the beneficial effect of MGN-3 on innate immune responses. These findings suggest MGN-3 incooperation into local wound dressings as a potential cost-effective therapeutic strategy to treat clinical DFU infections warrants further investigation.

P12

Antivirulence Compounds as Alternatives to Antibiotics: Repurposing aurodox as a treatment for Enterohaemorrhagic *E. coli*

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Abstract

Aurodox, a specialised metabolite from the soil bacterium *Streptomyces goldiniensis* was discovered in 1972 and originally was investigated for its anti-staphylococcal and streptococcal properties. However, aurodox has been recently identified from large-scale compound screens as an inhibitor of the Enteropathogenic *Escherichia co*li (EPEC) Type III Secretion System (T3SS). Therefore, to gain an understanding of its mechanism of action and to assess the suitability of this molecule for repurposing as an anti-virulence compound multidisciplinary approach to understanding aurodox was used. Whole transcriptome analysis, cell infection and GFP-reporter assays were used to demonstrate that aurodox transcriptionally downregulates the expression of the Locus of Enterocyte Effacement (LEE) pathogenicity island- which encodes for the T3SS, acting via its master regulator, Ler. We have also observed these effects across other enteric pathogens carrying a homologous T3SS such as Enterohemorrhagic *Escherichia coli* (EHEC). Significantly, unlike traditional antibiotics, aurodox does not induce the production of shiga toxin. The biosynthesis of aurodox by *S. goldiniensis* was also investigated. Sequencing the whole genome of S. goldiniensis enabled the identification of the putative aurodox biosynthetic gene cluster (BGC). We have cloned and expressed this gene cluster in multiple heterologous hosts including *Streptomyces coelicolor* M1152 and can confirm this BGC is responsible for aurodox production. In-depth analysis of the BGC supports a model of a polyketide synthase pathway involving a combination of both cis and trans-Acyltransferases which synthesise the aurodox polyketide backbone. Furthermore, multiple aurodox resistance genes at distinct loci have been identified and their role in resistance has been explored.

P13

The Pathogenesis of the Human Papillomavirus in Cancer of the Oropharynx

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Abstract

Background: Human papillomavirus (HPV)-mediated Oropharyngeal Cancer (OPC) is becoming increasingly more prevalent. The aetiology is not well established but is believed to possess a similar carcinogenesis to Cervical Cancer, whereby HPV infects traumatised epithelium in the oropharynx, such as the reticulated crypts within the palatine tonsil. We aim to investigate the natural history of HPV within the oropharynx and identify biomarkers that may influence disease prognosis.

Methods: We have established a clinical study with Derby, Burton, and Leicester hospitals, recruiting men and women over the age of 18 undergoing routine tonsillectomy. They consent to donate non-cancerous tonsils and answer a questionnaire consisting of questions on demographics, as well as behaviours known to be risk factors for oral HPV, including drinking and smoking habits, number of sexual partners and sexual risks. Analyses will determine correlations between HPV positivity with age, gender, and lifestyle.

Tonsils that are identified as HPV-positive by qPCR and Biobank-sourced OPC samples will undergo p16 immunohistochemistry for HPV detection, and HPV in situ hybridisation for confirmation of HPV positivity. Multiplex immunofluorescence will be performed using selected biomarkers including CK7, EGFR, PD-1, PD-L1, ER, CD8, HPV16/18 E6, and HPV16 E7 that are believed to influence disease prognosis.

Conclusions: The results of this study will prospectively characterise HPV infections within non-cancerous tonsils and describe the natural history of HPV within the oropharynx including the expression of biomarker targets. Through analyses of questionnaire data and biomarker expression, this will support clinical prognosis and influence treatment strategies for HPV-positive OPC.

P14

Investigating Immunological Cross-Reactivity Between Crimean-Congo Haemorrhagic Fever Virus and Nairobi Sheep Disease Virus

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Abstract

Immunological cross-reactivities between viruses of the *Nairoviridae* family have been reported within the literature, decreasing the analytical specificity of many common serological diagnostics (ELISAs, neutralisation tests). In the field, cross-reactivity is a problem in regions where nairoviruses co-circulate in ruminant populations, as it can lead to the misclassification of past heterologous viral infections from the same family.

Using Crimean-Congo haemorrhagic fever virus (CCHFV) and Nairobi sheep disease virus (NSDV) as a model, we aimed to quantify the effects of antibody cross-reactivities on IgG ELISAs, the most widely used serological assay for testing the presence of nairoviruses among livestock. Two in-house IgG ELISAs were developed for both CCHFV and NSDV, based on two antigens, the nucleoprotein (NP) and the glycoprotein C (Gc). By testing a large sheep sera sample set (n=1200) from a country where only CCHFV is reported to be endemic, we can confidently infer any resultant positivity on the heterologous NSDV ELISA to be the effect of immunological cross-reactivity.

When correlating OD values between our in-house CCHFV and NSDV Gc-based ELISAs, we found a significantly strong positive correlation, suggesting nairovirus antibodies to be particularly cross-reactive to the Gc antigen. Unlike the Gc antigen, the NP appears to be less cross-reactive, with no significant correlation when comparing CCHFV and NSDV OD positivity. In regions where nairovirus distribution ranges overlap, NP-based IgG ELISAs offer a more effective diagnostic tool to mitigate the effects of immunological cross-reactivity.

P15

The pathogenesis of HPV within oropharyngeal cancer

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Abstract

Background: Human papillomavirus (HPV)-positive oropharyngeal cancers (OPCs) of the base of the tongue and tonsils, are increasing. The prevalence of oral HPV infection within healthy individuals is reported as high as 35% and is linked to alcohol consumption, smoking, age and certain sexual behaviours with men at greater risk. Unlike HPV-driven cervical disease, there are few prospective studies of the pathogenesis of an oral HPV infection; thus, we aim to investigate the pathogenesis of HPV within the healthy oral mucosa, and to identify a panel of biomarkers that may indicate cellular changes initiated by a HPV infection.

Methods: We have established a clinical study with hospitals in Derby, Burton, and Leicester. Non-cancerous tonsils, a tonsillar swab, and a lifestyle questionnaire are collected from individuals undergoing routine tonsillectomy surgery. We have developed an HPV-DNA qPCR screen to determine HPV-status. qRT-PCR will be used to analyse viral type and load of HPV-positive specimens. Expression of viral genes E2, E6, and E7 will then be analysed to give an indication of the viral pathogenesis of an oral infection. SWATH mass spectrometry will be employed to identify a panel of protein biomarkers present in HPV-positive tissue.

Conclusions: Our study will describe oral HPV prevalence and pathogenesis within our cohort. Our preliminary data suggests a 22% prevalence. Through multivariate analysis of the questionnaire data and biomarker expression, the work has the potential to identify individuals at increased risk of HPV-mediated disease who would benefit from further intervention.

P16

Staphylococcus aureus manipulates the anti-inflammatory adenosine receptor, Adora2a, in primary human neutrophils to facilitate its intracellular survival by supressing neutrophil effector functions.

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Abstract

Staphylococcus aureus is a major pathogen, and its treatment is complicated by increasing multi-drug resistance. This bacterium's propensity for intracellular survival presents a further challenge, with significant evidence suggesting *S. aureus* can hijack phagocytes for host dissemination leading to worse clinical outcome. Host pathways that facilitate intracellular survival in phagocytes remain unclear.

To identify novel therapeutic targets engaged by *S. aureus* to survive intracellularly within primary human neutrophils, a multiplex gene expression analysis was performed using the Nanostring nCounter platform and RNA isolated from neutrophils harboring viable intracellular *S. aureus*.

Pathway analysis revealed upregulation of multiple host pathways, the most significantly being cytokine and growth factor signaling pathways. Among growth factor signaling associated genes, the anti-inflammatory adenosine receptor, Adora2a (A2A) was significantly upregulated along with several other genes related to A2A activation. A2A expression was confirmed to be significantly upregulated at the gene and protein level in neutrophils harboring intracellular *S. aureus*, 1h & 3h post infection. Importantly treatment with an Inhibitor of A2A, ZM 241385, significantly reduced intracellular survival of *S. aureus* within neutrophils and was associated with enhanced ROS and myeloperoxidase expression. A2A inhibition also enhanced neutrophil production of the pro-inflammatory cytokine IL-8 whilst limiting expression of the anti-inflammatory cytokine IL-10.

Taken together, our work suggests that *S. aureus* targets A2A signaling to enhance intracellular survival in neutrophils by suppressing their effector functions and potentially skewing them towards a more anti-inflammatory phenotype and reveals a potential new therapeutic target to overcome the intracellular survival of *S. aureus*.

P17

Enhancing an artificial urine for culture of uropathogenic *Escherichia coli* and *in vitro* gene expression studies

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Abstract

Uropathogenic *Escherichia coli* (UPEC) are the most common cause of urinary tract infections (UTIs), which pose a great burden to global health and the economy. While antimicrobial therapy is successful in some individuals, there are increasing numbers of antimicrobial resistant UPEC strains. There is a clear need for novel therapies and strategies to tackle UTI. To meet this need, we must first better understand UPEC physiology and gene expression. The challenges associated with the reproducibility and storage of pooled human urine (PHU) mean that artificial urine is an attractive option for *in vitro* studies of UPEC strains. Unfortunately, these artificial urine formulations may contain components not always present in healthy human urine, making them unsuitable alternatives to human urine for gene expression studies. We have performed growth studies showing that a multipurpose artificial urine does not support optimal growth of UPEC strains CFT073 or UTI89. We have also shown, using liquid chromatography mass spectrometry, that the multipurpose artificial urine has a different metabolic profile to PHU. We have modified the multipurpose artificial urine using metabolites identified by LC-MS of PHU. Our modified artificial urine better supports the growth of UPEC strains and induces a similar transcriptome to that induced by culture in PHU. Further work will be needed to modify the artificial urine to generate a more reliable and reproducible alternative to PHU for *in vitro* culture and gene expression studies of UPEC.

P18

Characterising the effects of aurodox as an antivirulence compound against Salmonella Typhimurium

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Abstract

One method to combat antimicrobial resistance is to employ the use of antivirulence compounds – treatments which prevent pathogenic bacteria from harming their host without otherwise interfering with survival. *Salmonella* spp. are Gram-negative bacteria and the causative agents of disease ranging from self-limiting gastrointestinal symptoms to severe, disseminated and potentially lethal illness. Partly responsible for *Salmonella'*s ability to cause disease are a pair of Type-3-Secretion Systems (T3SS) encoded on pathogenicity islands – Salmonella pathogenicity islands (SPI) 1 and 2, which respectively enable cellular invasion and intracellular persistence/growth. Previous work in our group has begun to characterise the mechanism of action of aurodox, a small molecule derived from *Streptomyces goldiniensis,* and a potent T3SS inhibitor in Enterohaemorrhagic *Escherichia coli* which exerts its effect by suppressing transcription of the LEE pathogenicity island.

Here we sought out to test if this antivirulence effect carries over to other enteric bacteria by asking if aurodox can also inhibit Type 3 Secretion in *Salmonella* Typhimurium SL1344. RT-qPCR suggested that aurodox suppressed transcription of the SPI-2 effector gene *sseB* but not the SPI-1 effector *sipC.* Transcriptional reporters for SPI-1 and SPI-2 further support the hypothesis that aurodox specifically inhibits SPI-2 mediated Type-3 Secretion in *Salmonella* Typhimurium. Growth kinetics in the presence and absence of aurodox suggest that aurodox inhibits growth of Salmonella Typhimurium in conditions that mimic the phagolysosome in which SPI-2 is expressed, but not in rich media. Taken together, this work sheds further light on the mechanism of action of a candidate novel antivirulence compound.

P19

Tissue Resident Memory (TRM) cells are generated in the respiratory tract of *Staphylococcus aureus*-colonized mice.

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Abstract

Anti-microbial resistance is a major obstacle in treatment of *Staphylococcus aureus* (SA) infection. The development of an effective vaccine remains a priority. Asymptomatic SA-carriage in healthy individuals potentially confers an immunological advantage during invasive infection, however, immune phenotypes imprinted by SA-colonisation may impact vaccine function. More in-depth understanding of immune responses to SA-colonisation is required.

TRM cells, maintained at barrier surfaces, are primed by antigen exposure for rapid recall memory responses to the same/novel pathogens. This study will access if SA-colonisation expands nasal tissue (NT) TRM cells, and the potential consequences for subsequent infection and/or vaccination.

Mice were intra-nasally SA-colonised. CD4+ and γδ+ TRM populations within the NT, were identified as CD4+CD69+CD44+CD62L-CD45- and γδ+CD44+CD27-CD45- following i.v. administration of fluorescent CD45 10mins before sacrifice. These cells produced IL-17 and were expanded up to 28 days post-colonisation, by which time bacteria had been cleared. Re-exposure of SA-colonized mice to SA amplified NT IL-17+ CD4+ and γδ+ TRM cell responses. This was independent of circulating lymphocyte recruitment, as FTY720 administration which inhibited lymphocyte egress from lymph nodes, did not inhibit TRM cell expansion or cytokine production. Interestingly, intra-nasal LPS administration to SA-colonized mice 28 days post-colonization also activated IL-17+ CD4+ and γδ+ NT TRM cells, suggesting a non-specific memory response could be generated. *Klebsiella pneumoniae* re-challenge also induced IL-17+ γδ+ NT TRM cells.

This data suggests immune exposure induced by SA-colonisation can induce tissue resident immune responses which enhance the host’s ability to deal with unrelated invasive respiratory pathogens.

P20

The Effects of Amino Acids on Bacterial Colibactin Expression

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Abstract

Some *Escherichia coli* strains of the B2 phylogroup synthesise a potent genotoxin, named colibactin, encoded for on their genomic *pks* island. In eukaryotic cells, colibactin induces DNA damage, chromosomal instability, and cell-cycle arrest, and it has been shown to trigger the development of colorectal cancer. Our results demonstrate the inhibitory effect of several amino acids on the *pks* island in colibactin-producing strains. All proteinogenic L-amino acids and corresponding D-enantiomers were tested for their ability to modulate *clbB* transcription through a p*clbB*-GFP reporter-assay in four different types of growth media. The most inhibitory amino acids were further validated by RT-qPCR. D-Serine, amongst the most repressive amino acids, was selected for further experiments in two colibactin-producing strains, CFT073 and Nissle 1917. We observed that repression of colibactin by D-Serine reduces the cytopathic responses normally observed during infection of HeLa cells with *pks*+ strains. Levels of γ-H2AX (a marker of DNA double strand breaks) were reduced 2.75-fold in cells infected with D-Serine treatment; and exposure of *pks*+ *E. coli* to D-Serine during infection caused a reduction in cellular senescence observable at 72 hours post infection. The association between *pks*+ commensal *E. coli* and colorectal cancer emphasises the need for colibactin-targeting therapeutics. We show that D-Serine has the potential to prevent colibactin-associated disease and that several other amino acids may be considered for further investigation as therapeutic candidates.

P21

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 take a combined approach of phage isolation, bioinformatic analysis of phage and prophage genomes and ultimately cloning, expression and analysis of potential tail-associated lysins (TALs). The focus is mainly on Staphylococcal spp. but also other DFU isolates collected from patients during the study. Phage genomes were obtained from NCBI virus database and examined using RAST webserver. In addition, phages are being isolated from human wastewater and agricultural waste, while DFU isolates are being sequenced and analysed using tools such as PHASTER to identify prophage contained therein. Although the research is still in progress, TALs with domains such as endopeptidase, lytic transglycosylase and amidase have been identified. This work will allow for genetic engineering of such endolysins for both detection and control of staphylococcal pathogens.

P22

Impact of vaccination on antibiotic resistance and population structure of *Salmonella* Typhi in Harare, Zimbabwe

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Abstract

Typhoid fever, caused by the bacteria *Salmonella enterica* serotype Typhi, results in 26 million infections each year. The emergence and global spread of multi-drug resistant (MDR) and fluoroquinolone resistant *S*. Typhi is a major concern, as it compromises treatment options. Vaccines are increasingly viewed as a key tool in the fight against AMR, both by preventing infection with a resistant pathogen (direct) or reducing the need for antimicrobial treatment (indirect).

In this project, our key goal is to investigate the impact of the 2019 typhoid conjugate vaccine campaign in Zimbabwe on circulating *S*. Typhi population structure and antibiotic resistance.

S. Typhi isolates were collected between 2012 and 2020 and included 92 pre-campaign and 152 post-campaign samples. Bacterial population structures were analysed using Illumina sequencing, and changes in phenotypic and genotypic resistance were explored, using software including Shovill, Prokka, Roary, Scoary, Mykrobe, and Genotyphi.

Following the campaign there was an overall reduction in typhoid cases in the populations vaccinated. We found no changes in the bacterial population structure or in measured phenotypic or genotypic resistance over time or in comparison to the non-vaccinated adult population.

While effective typhoid vaccination is likely to reduce the overall disease burden in the population, there is no evidence of short-term alteration to the profile of *S.* Typhi causing human infection.

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