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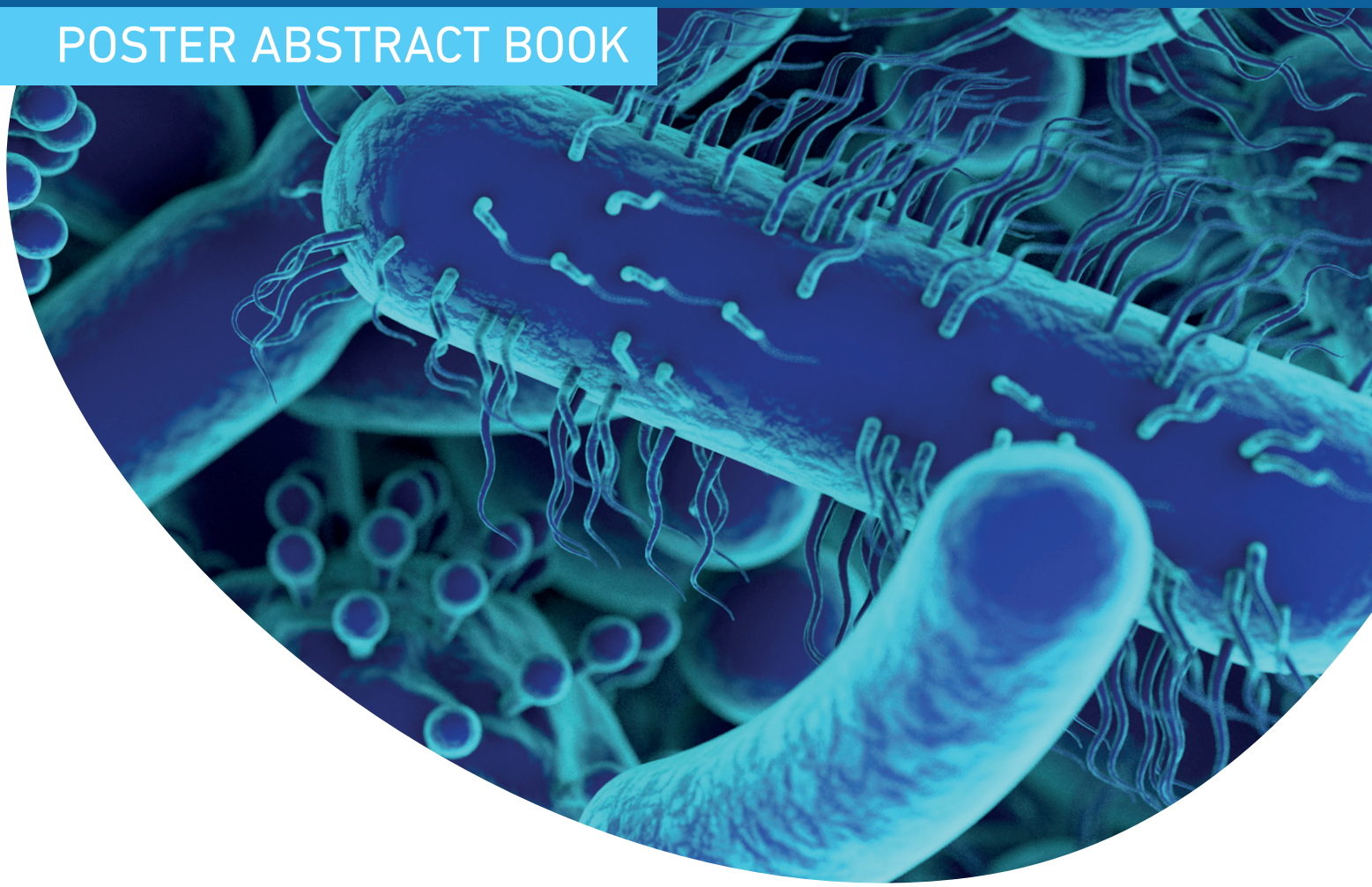
POSTER ABSTRACT BOOK



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P01

The human skin bacteria Staphylococcus epidermidis fermentation end-product ameliorates UVB-induced ROS generation through the production of free electron transfer

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Abstract

UVB-induced skin damage results in various inflammatory disorders through the induced generation of reactive oxygen species (ROS) that quickly inundate tissue antioxidants and in severe cases it can lead to skin cancer. To investigate efficacies of human skin commensal bacteria *S. epidermidis (ATCC12228)* with its specific skin fermentation inducer (SFI), named as *Ncueh1* that on fermentation produces antioxidant electrons against UVB induced skin damage. *In vivo* affirmation on ICR mice has confirmed the photoprotective role and maintained sufficient Anti-4 hydroxynonenal (4-HNE), a major biomarker of oxidative stress and lipid peroxidation, has been recognized as an important molecule in UVB-induced skin damage. Results in the Western blot analysis using antibodies to 4-HNE demonstrate that 4-HNE is induced in mouse skin overexposure to UVB. On the topical application of *S. epidermidis (ATCC12228)* plus its specific prebiotic *Ncueh1* onto mouse skin before and after UVB exposure significantly reduced the UVB-induced 4-HNE. Application of *S. epidermidis (ATCC12228)* alone or prebiotic *Ncueh1* alone does not influence the level of 4-HNE in UVB exposed skin. Electrochemical behavior of *S. epidermidis (ATCC12228)* with and without *Ncueh1* has been determined to produce electron transfer this result suggests that electrogenic and antioxidant property of *S. epidermidis (ATCC12228). We have also tested the similar efficacy from the human skin isolated S. epidermidis to prove the similar function of bacteria in compared to ATCC12228 Strain* that can mediate prebiotic *Ncueh1* to produce free electron which may effectively neutralize and scavenges the formation of free radical by UVB irradiation.

P02

Defining the link between efflux pumps and biofilm formation

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Abstract

Biofilms are intrinsically important to our understanding of bacterial pathogens. The majority of bacterial life exists as part of a biofilm, as despite this, we have very little understanding of the genetic pathways that facilitate and drive biofilm formation. Work in our group has outlined a phenotypic link between efflux pump activity and biofilm formation, both of which have been implicated in decreased susceptibility to multiple antibiotics. Genetic or chemical inactivation of efflux results in a substantial decrease in biofilm formation. We have determined that this is due to transcriptional repression of one of the main components of the biofilm: the amyloid fibrous protein, curli. This relationship between efflux activity and biofilm formation has been identified in many Gram-negative and Gram-positive bacterial pathogens, but the regulatory network through which these phenotypes are linked is unknown. My PhD project aims to determine the pathway that links efflux pump activity and biofilm formation. I will investigate this using TraDIS, which is a large-scale transposon mutagenesis approach that will be used to determine all of the genes responsible for efflux activity and biofilm formation. My poster will present preliminary results from this genome-wide screen in *E. coli*. I will outline the further work to be undertaken, including repeating this experiment in *Salmonella* Typhimurium and formulating and testing hypotheses as to how efflux activity and biofilm formation are linked in both species. This will overall improve our understanding of important mechanisms of antimicrobial resistance in human pathogens.

P03

Carbapenem-Resistant Enterobacteriaceae :A serious concern in cancer patients

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Abstract

INTRODUCTION: Gram-negative bacteria, including *Enterobacteriaceae*, are an important cause of infections in cancer patients. Members of *Enterobacteriaceae* are commonly isolated from bloodstream infections, surgical site infections, urinary tract infections, and lower respiratory tract infections. There has been an increase in the isolation of gram-negative MDRO's over the years with ESBL's and for the last few years with carbapenem-resistant *Enterobacteriaceae* (CRE). CRE's have become a serious threat to cancer patients.

MATERIAL & METHODS : A total of 61331 clinical samples from 19015 patients were received in the Dept of Microbiology, Tata Memorial Center, Mumbai, India during January 2018 to May 2019. All the samples were processed as per routine microbiological procedures and antimicrobial susceptibility testing was performed as per CLSI guidelines.

RESULTS : Blood was the commonest sample received followed by urine, respiratory tract samples, surgical site samples and sterile body fluids. *E. coli* was the commonest microorganism isolated followed by *Klebsiella pneumoniae*, *P. aeruginosa*, *S.aureus, Acinetobacter sp*, *Enterobacter* sp and *Enterococci.* Colistin was the most susceptible antibiotic for gram negative organisms followed by tigecycline, aminoglycosides, cefoperazone-sulbactam and piperacillin-tazobactam. Overall 77.8% CRE's were detected whereas it was 81.8% in LRTI, followed by 82.5% in UTI, 79.1% in BSI and 75% in SSI.

CONCLUSION: The increasing prevalence of CRE infections represents a major threat to cancer patients. With the high mortality of CRE infections and increasing resistance to available antibiotics, it is urgent for the medical community to develop new and effective therapeutic strategies. Robust anti-microbial stewardship is the need of the hour.

P04

The (p)ppGpp-binding GTPase Era promotes rRNA processing and cold adaptation in *Staphylococcus aureus*

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Abstract

Ribosome assembly cofactors are widely conserved across all domains of life. One such group, the ribosome-associated GTPases (RA-GTPase), act as molecular switches to coordinate ribosome assembly. We previously identified the *Staphylococcus aureus* RA-GTPase Era as a target for the stringent response alarmone (p)ppGpp, with binding leading to inhibition of GTPase activity. Era is highly conserved throughout the bacterial kingdom and is essential in many species, although the function of Era in ribosome assembly is unclear. Here we show that Era is not essential in *S. aureus* but is important for 30S ribosomal subunit assembly. Protein interaction studies reveal that Era interacts with the 16S rRNA endonuclease YbeY and the DEAD-box RNA helicase CshA. We determine that both Era and CshA are required for growth at suboptimal temperatures and rRNA processing. Era and CshA also form direct interactions with the (p)ppGpp synthetase Rel_{Sau}, with Rel_{Sau} positively impacting the GTPase activity of Era but negatively affecting the helicase activity of CshA. We propose that in its GTP-bound form, Era acts as a hub protein on the ribosome to direct enzymes involved in rRNA processing/degradation and ribosome subunit assembly to their site of action. This activity is impeded by multiple components of the stringent response, contributing to the slowed growth phenotype synonymous with this stress response pathway.

P05

Staphylococcus aureus targets corneodesmosin to colonise skin in atopic dermatitis

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Abstract

Staphylococcus aureus plays an important role in atopic dermatitis (AD), a chronic inflammatory skin disease. Skin colonization by *S. aureus* exacerbates the clinical severity of AD. However the molecular determinants of adhesive interactions between bacteria and skin are poorly understood. Elucidating the molecular basis of skin colonization is crucial to understand the pathogenesis of AD and to develop new therapeutic strategies. *S. aureus* adheres to dead flattened skin cells known as corneocytes in the outermost layer of the epidermis. Corneocytes in AD skin have an altered surface morphology compared to corneocytes from healthy skin. Corneodesmosin, an adhesive protein normally confined to the tight junctions between corneocytes, decorates the tips of villus-like projections on the surface of AD corneocytes. Here we identify corneodesmosin as a key ligand for *S. aureus* on AD corneocytes. We show that strain of *S. aureus* isolated from infected AD skin lesions adhere to recombinant corneodesmosin. Using surface plasmon resonance, we show that three cell wall anchored proteins expressed recombinantly bind to corneodesmosin with high affinity. Two of these proteins promote *S. aureus* adherence to corneodesmosin. High-resolution imaging of corneocytes from AD skin revealed that strong adhesive interactions are not uniformly distributed across the corneocyte surface but mostly concentrate on the tips of villus-like projections, consistent with corneodesmosin being a ligand. In summary this study identifies novel interactions between *S. aureus* and corneodesmosin and thus provides important new insights into the first steps in the establishment of *S. aureus* skin colonisation in AD patients.

P06

An Acidic Environment Drives *Mycobacterium tuberculosis* to Selectively Adopt a Lipid Rich Diet

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Abstract

Mycobacterium tuberculosis (Mtb) is an intracellular pathogen that thrives inside macrophages despite the presence of immune-driven bactericidal conditions such as acidic pH. However *in vitro,* growth of *Mtb* is greatly attenuated even at mild acidic pH (<5.8). We have observed that fatty acids promote *Mtb* growth at a pH lower than ever described before *in vitro* and corresponding to the pH found inside phagolysosomes (< pH4.9). Using genetic and metabolomics approaches, we investigated the hypothesis that acidic pH drives *Mtb* to preferentially utilize fatty acids (a host-relevant carbon source for *Mtb*) over gluconeogenic carbon sources to support growth. We found that the survival of *Mtb* lacking genes known to be required for growth on fatty acids as sole carbon sources was severely reduced in a pH-specific manner even when both glycerol and fatty acids are available. Furthermore, we applied metabolomics using 13-C labelled glycerol and oleic acid to trace the preferred incorporation of oleic acid over glycerol in acidic conditions. Our data has revealed a pH-driven carbon utilization program whereby *Mtb* selects host relevant carbon sources to fuel its growth. Here, we demonstrate that in addition to the presence and the availability of particular nutrients at the site of infection, physicochemical conditions such as low pH can regulate their utilization by pathogens.

P07

The antibiofilm effects of docosahexaenoic acid.

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Abstract

The ability of bacteria to form biofilms allows for recalcitrance against conventional antibiotic therapies (Potera, 1999). Therefore, this has contributed to the prevalence of biofilm acquired infections (BAI) clinically (Percival and Kite, 2018) which has resulted in increased morbidity and mortality amongst patients, with immunocompromised patients at risk in particular (Donlan, 2002). Biofilm formation on abiotic and biotic surfaces has enabled bacteria to colonise major organs and medical devices, such as within the lungs, on implant surfaces, contact lenses, and urinary catheters (Vinh and Embil, 2005; Percival *et al.*, 2012; Seth *et al.*, 2012). Docosahexaenoic acid (DHA) is a poly-unsaturated fatty acid known to exhibit antibiofilm and antimicrobial effects (Sun *et al.*, 2017; Kim *et al.*, 2018). Our research has found that DHA possesses strong anti-biofilm effects against *Klebsiella pneumoniae* and *Enterococcus faecalis* at low mM concentrations. DHA was capable of reducing biofilm formation by both *K. pneumoniae* and *E. faecalis* by approximately 60%. It is believed that this is the first time these effects have been reported. We have also have evidence that DHA in conjunction with an antibiotic is better at reducing biofilm formation by these strains better then either alone.

To date, there is much debate into how DHA exerts these anti-biofilm effects. DHA has been reported to distort bacterial membrane’s therefore, impacting biofilm formation (Sun *et al.*, 2017). We aim to elucidate the precise mechanism of action by utilising a number of genetic approaches alongside the dynamic flow cell system and confocal microscopy

P08

Antimicrobial resistance in bacteria associated with childhood pneumonia in Malawi

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Abstract

Community acquired pneumonia (CAP) is a disease caused by an infection (by bacteria, viruses or fungi) that in cases of chronic infection lead to high mortality rates particularly in children. Pneumonia is the leading cause of death in children under the age of five worldwide with over 2 million deaths reported in 2016 by the World Health Organisation. Developing countries in sub Saharan Africa such as Malawi see some of the highest rates of the disease. CAP infections are responsible for 20% of deaths of new-borns in Malawi. Antibiotic resistance in disease causing organisms is of growing concern in countries like Malawi.

We obtained bacterial isolates from blood samples taken from Malawian children, aged 18 -60 months, diagnosed with CAP. *Streptococcus pneumonia* is the primary pathogen in CAP but associated ESKAPE pathogens like *Pseudomonas* and *Staphylococci spp and Escherichia coli* are of growing importance. These were the most common bacteria purified, isolated and characterised from these samples resulting in a unique culture collection. These pathogens have been screened for antimicrobial resistance with a variety of antibiotics to generate a resistance profile based on the minimal inhibitory concentrations of selected isolates using antibiotic susceptibility disks and by broth dilution method. Initial assessment shows current front-line therapies used by the Malawian ministry of health are ineffective in treating CAP.

Our research aims to fully characterise these strains at the genetic level to better understand the mechanisms behind how antibiotic resistance develops to aid in combating this growing issue in countries like Malawi.

P09

Investigating the host-pathogen interactions during nosocomial methicillin resistant *Staphylococcus aureus* pneumonia

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Abstract

Pneumonia accounts for more deaths than any other infectious disease worldwide. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common causes of hospital-acquired pneumonia. The prevalence of MRSA is increasing worldwide. To confront the growing problem of MRSA, we require a greater understanding of the host-pathogen interactions during infection. This remains poorly understood partly due to the lack of *in vivo* models relevant to infections occurring in healthcare settings. Most research to date has focused on highly virulent and cytotoxic MRSA strains, despite the fact that many nosocomial infections are caused by MRSA isolates that exhibit low cytotoxicity in *ex vivo* models and low virulence in mice. This study aims to functionally dissect host- and bacterial-directed mechanisms that lead to mortality in nosocomial settings. We have developed a nosocomial murine model of antibiotic conditioning, which we found lowers the barrier to infection, mimicking the nosocomial setting. We demonstrate that exposure to antibiotics mitigates the impact of reduced bacterial virulence in mice, allowing for a permissive environment for hospital adapted HA-MRSA isolates. These findings establish a robust model that is enabling us, for the first time, to probe the bacterial and host factors important during nosocomial infections, which are relevant to hospitalized patients that are also subjected to antibiotic regimes.

P10

Significance of the chromosomal positions of the genes encoding DNA gyrase in *Salmonella enterica* serovar Typhimurium.

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Abstract

Changing positions of genes on a chromosome is an informative way of learning about why an existing chromosome structure and gene order was selected by evolution in bacteria. In *Salmonella gyrA* and *gyrB* – genes encoding DNA gyrase – enzyme that introduces negative supercoils in DNA, are located almost at opposite poles of a chromosome. However, in many other bacteria they are arranged in a *gyrBA* operon. In order to investigate the significance of this fact, *gyrBA* operon was made in *Salmonella* by bringing *gyrA* open reading frame and its ribosome binding site directly downstream of *gyrB* and under a control of its regulatory regions, the original copy of *gyrA* was deleted. The *gyrBA* strain obtained as a result exhibits no differences from the wild type in growth and morphology, however, the ability to supercoil DNA is altered between *gyrBA* and the WT. This is specifically important at conditions mimicking environment inside a macrophage in terms of Mg^{2+} concentration, as it may suggest alteration of *gyrBA* survival inside a macrophage. An attempt to make strain with *gyrAB* operon was not successful, as it was not possible to delete the original *gyrB*, suggesting particular importance of its position.

P11

Understanding Selection of Antimicrobial Resistance in Biofilms through Experimental Evolution

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Abstract

Background

Biofilms possess several important properties which differentiate them from free-living cells, including their response to selective pressures. This work demonstrates a link between exposure to non-therapeutic antimicrobials and selection for antibiotic resistance in experimentally evolved biofilms of *Pseudomonas aeruginosa*.

Methods

Biofilms of *P. aeruginosa* PA14 were experimentally evolved through successive subculture under periodically increasing antimicrobial stress. Beginning at 0.25-times MIC, biofilms were exposed to benzalkonium chloride, ciprofloxacin, colistin, zinc or copper sulfate. When the antimicrobial stress resulted in growth failure, the last successful passage was phenotyped. Biofilm formation was quantified via crystal violet uptake and MICs determined by broth microdilution according to EUCAST guidelines.

Results

Experimental evolution selected for mutants which produced approximately four-fold more biomass than the parenteral strain. Both ciprofloxacin and chloramphenicol readily selected for mutants able to survive >1024-times MIC. Conversely, no change in susceptibility was observed for either metal. However, despite zinc sulfate not selecting for decreased susceptibility to itself, cross-resistance to both colistin and meropenem was observed in the zinc-passaged lineages. Benzalkonium chloride did not select for a significant change in susceptibility when grown planktonically. However, when assayed as a biofilm, a 5-fold decrease in benzalkonium chloride susceptibility was observed, indicating a biofilm-specific mechanism of antimicrobial tolerance.

Conclusion

Mutants with an increased capacity to form biofilms and decreased antimicrobial susceptibility were successfully selected. Furthermore, zinc sulfate, a common feed additive, was able to select for decreased susceptibility to clinically-relevant antibiotics, lending to concerns that ubiquitous non-therapeutic antimicrobials may act as non-canonical drivers of antibiotic resistance.

P12

INFLUENCE OF PROBIOTICS ON *CRONOBACTER SAKAZAKII* VIRULENCE

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Abstract

Cronobacter sakazakii are Gram-negative, facultative anaerobic bacteria of the family *Enterobacteriaceae* that have been implicated in rare but severe cases of illnesses predominantly in premature and new-born infants. This opportunistic pathogen has been isolated from clinical as well as from a range of food and environmental sources. Especially in new-born children, *C. sakazakii* infections have been epidemiologically linked to the ingestion of powdered infant formula (PIF).

To reduce the risk of infection, probiotics should be tested as an addition to PIF. Therefore, the inhibitory effect of different probiotic strains on *in vitro* growth of *C. sakazakii* and the ability to colonize human intestinal epithelial cells (Caco-2 and HT-29) was analyzed. The results show that co-infection with probiotic microorganisms protected the eukaryotic epithelial cells from invasion of the pathogen in a probiotic strain-dependent manner.

Furthermore, with regard to the prophylactic effectiveness of probiotics, the inhibitory effect on *C. sakazakii* growth of sterile supernatants from probiotics in different PIF will be examined in order to developed approaches for safe and effective use of probiotics in PIF.

P13

Fibronectin Binding Protein B Mediates Adherence of Staphylococcus aureus to the Corneocyte Protein Loricrin

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Abstract

Staphylococcus aureus colonizes multiple body sites, primarily the anterior nares, skin and nasopharynx. Targeted decolonisation with topical antibiotics is used to prevent infection in specific groups but the efficacy of this approach is threatened by antibiotic resistance. Advances in understanding how *S. aureus* establishes colonization are needed to inform new strategies for the elimination of *S. aureus* carriage in risk groups. Loricrin-deficient knock-out mice were previously found to be more resistant to nasal colonization by *S. aureus* than wild-type mice suggesting that this cornified envelope protein is the main ligand recognised by *S. aureus*. Previous studies showed that the cell wall anchored (CWA) protein ClfB promotes adherence of *S. aureus* to loricrin. Here we show that mutation of *clfB* in a set of clinical isolates does not completely abolish adhesion to loricrin. *In vitro* adhesion assays revealed that the CWA fibronectin binding protein B (FnBPB) can also mediate adherence to loricrin. Null mutations in the *fnbB* gene in clinical strains reduced their adherence to loricrin. Double *clfB* and *fnbB* mutants completely lost the ability to adhere to loricrin. Surface Plasmon Resonance was used to demonstrate that recombinant FnBPB binds to loricrin and to identify the region of the FnBPB protein containing the binding site. In summary this study identifies a novel interaction between fibronectin binding proteins and loricrin and provides important new insights into the repertoire of *S. aureus* proteins facilitating adherence during colonization.

P15

A mobile genetic element-encoded copper hypertolerance locus promotes resistance of MRSA to copper toxicity and killing within phagocytes

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Abstract

Pathogens are exposed to toxic levels of copper during infection and copper tolerance appears to be a general virulence mechanism used by bacteria to resist host defences. *Staphylococcus aureus* is a leading human pathogen causing a range of infections ranging from minor skin and soft tissue infections to serious bacteraemia. Mobile genetic elements carrying copper tolerance genes are widespread among clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) globally. Here we show that a mobile genetic element-encoded copper hypertolerance locus, *copB-mco* promotes tolerance of MRSA to copper in vitro, and to killing within primed macrophages and in human phagocytes. The *copB* and *mco* genes encode a copper efflux pump and a putative multicopper oxidase, respectively. Isogenic mutants lacking *copB* or *mco* had impaired growth in subinhibitory concentrations of copper. Mutation of the *mco* gene reduced the ability of MRSA to survive incubation in a solution of copper chloride, indicating that Mco is required to resist copper toxicity. Isogenic *copB* and *mco* mutants were impaired in their ability to persist intracellularly in macrophages and were less resistant to phagocytic killing in human blood than the parent strain. Our findings suggest that the gain of mobile genetic elements carrying copper hypertolerance genes contributes to the emergence of virulent strains of MRSA, better equipped to resist killing by immune cells. Future work will focus on understanding the molecular mechanisms underlying copper hypertolerance in MRSA and the biological consequences of acquisition of copper hypertolerance.

P16

The evolution of *Pseudomonas aeruginosa* during short-term acute respiratory infections

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Abstract

Background: The emergence and spread of bacteria with resistance to multiple antimicrobial agents is raising the mortality rates associated with bacterial infections, and poses a fundamental threat to human health. *Pseudomonas aeruginosa* is an opportunistic human pathogen that is responsible for 10-15% of health-care associated infections worldwide, and is known for its ability to rapidly develop enhanced resistance during the course of treating an infection.

Methods: *P. aeruginosa* isolates have been collected from ICU patients in hospitals across Europe over a longitudinal sampling of short-term acute respiratory infection. Twelve isolates per patient per sampling of infection have been acquired. Genome sequencing and high-throughput phenotyping of growth rate and antibiotic resistance profile has been carried out.

Results and conclusions: Using high-throughput phenotyping and genome sequence data we have been able to characterise population diversity and variation in phenotypic resistance profile, growth rate and acquired resistance gene content of these isolates. A gradient of diversity was found within patient infections, ranging from monoclonal to multiple strains present. Large within-patient population diversity in phenotypic resistance profile and acquired resistance gene content could be observed in multiple sequence-type infections.

This research has been carried out in collaboration with the COMBACTE-MAGNET research consortium (Combatting Bacterial Resistance in Europe – Molecules against Gram-Negative Infections).

P17

Utilization of ethanolamine by *Listeria monocytogenes*: a mechanism with potential relevance for gut adaptation

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Abstract

Listeria monocytogenes (*Lm*) is an intracellular bacterial pathogen that causes listeriosis, a severe food-borne infection in humans. To cause infection, *Lm* can cross major physiological barriers, the first being the intestinal barrier. During gut infection, *Lm* must adapt to the gastrointestinal environment and compete with the resident microbiota. Recent evidence indicates that the utilization of ethanolamine potentially contributes to survival in the gut. To investigate ethanolamine utilization by *Lm*, two knockout mutants for the ethanolamine ammonia lyase subunit-coding gene *eutB* and *eutM*, coding a putative structural component of the ethanolamine bacterial microcompartment, were constructed from *Lm* EGDe wild type strain. We show that *Lm* is able to utilize ethanolamine as a nitrogen source but not as a sole carbon source and that *eutB* is necessary for full ability to utilize ethanolamine. *In vitro* assays with a human epithelial C2BBe1 cell-line suggest *eutB* and *eutM* might have a role in cell invasion, a key step in the *Lm* infectious cycle, as the entry of both mutant strains is impaired. By unravelling the mechanisms of ethanolamine utilization by *Lm*, this work provides important insights on how *Lm* adapts to the environment and to the host, a factor that may be important during infection of the gut.

P18

Plasma activated liquids: New decontamination solutions

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Abstract

In the field of new decontaminants, there is an increasing consensus that improved disinfection of environmental surfaces is needed in patient care facilities as an important component in the overall strategy for prevention of HAI. The interaction of cold atmospheric plasma, i.e. an ionized gas, with liquids results in complex physical and chemical processes, which offer a source of short-lived and long-lived reactive chemical species that are critical for microbial inactivation. These solutions may fulfil the urgent need for new decontaminant solutions for special purposes such as disinfection of surfaces, or use as an antiseptic for body surfaces.

In this study, we explored the bactericidal effects of plasma activated liquids (PALs) on *E.coli* and *S.aureus* strains and investigated factors which influence PALs stability over time. Liquids of interest were non-complex solutions such as water and saline. An atmospheric cold plasma system using air was employed for the generation of PALs. The solutions were compared with respect to their content of long-lived reactive chemical species and bactericidal effects.

Our results documented that PALs may carry different concentrations of chemical species and maintain diverse antimicrobial properties. The bactericidal activity of these solutions demonstrated high thermal stability and could be preserved over a 6 month period through specific sub-ambient storage conditions. Investigation of the inactivation processes in relation to the PALs' chemical composition will enhance our knowledge on how prokaryotic and eukaryotic cells respond to these, and demonstrate how PALs could be a promising treatment method for future applications, with chemical and antimicrobial stability.

P19

A matter of life and death; identifying host genomic factors that determine susceptibility of chickens to highly pathogenic avian influenza

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Abstract

DNA samples collected from survivors of recent outbreaks of highly pathogenic avian influenza (HPAI) in Mexico and the USA have provided a rare opportunity to study the genetic mechanisms underpinning susceptibility of chickens to this devastating and economically impactful disease which normally exhibits 70-100% mortality in the chicken host. Whole Genome Sequence (WGS) data has been used to perform Genome-Wide Association Studies (GWAS), which have highlighted single nucleotide polymorphisms (SNPs) segregating significantly between survivors and controls, with a pedigreed experimental group exhibiting a highly significant signal on chicken chromosome 2 in the region of a biologically relevant gene. Candidate SNPs for resilience are currently being validated using *in vitro* gene editing methodologies that modulate candidate gene expression to investigate the effect on viral replication and cellular response to HPAI infection. A detailed understanding of the genomic resilience to HPAI from this study will have implications for both the poultry industry and for public health.

P20

CHARACTERISING THE URINARY MICROBIAL COMMUNITY IN PATIENTS WITH SYMPTOMS OF URINARY TRACT INFECTION

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Abstract

BACKROUND

Every year 250 million individuals develop urinary tract infections (UTI), the diagnosis of which, usually involves the ‘gold standard’ midstream urine culture (MSU) test. Prompt detection aided by sensitive tests may increase the likelihood of successful treatment. However, recent evidence highlights the potential inadequacies of this test.

METHODS

We assessed the UK’s MSU culture by comparison with enhanced culture techniques and 16S rRNA gene sequencing. With ethical approval, patients attending their first consultation at the Whittington Hospital Clinic and asymptomatic controls provided MSU specimens. Each specimen was submitted to the Hospital Microbiology laboratory for MSU culture. 16S rRNA gene sequencing was performed on 1ml unspun urine and 30ml of centrifuged urine.

RESULTS

Urine specimens were analysed for 33 patients (mean age= 49 years, sd=16.5) and 29 controls (mean age=40.7 years, sd=15.7). Comparison of routine MSU cultures between patients and controls indicated that the test failed to discriminate between patients and controls (χ^2 = 0.539, df = 1, P = 0.674). Bacterial DNA was detected in 97.0% patients and 89.7% controls. *Enterobacteriaceae* was most abundant in patients, whereas *Streptococcus* dominated in controls. A higher distribution of the median number of observed taxa (centrifuged and unspun samples combined) was seen in patients (χ2 = 8.0, df = 2, P <0.05).

CONCLUSION

The MSU culture failed to discriminate between patients and controls and missed recognised uropathogens that were detected with bacterial DNA sequencing. Microbial communities characterised by sequencing warrant further investigation to understand the role of each member in UTI.

P21

Vorinostat (SAHA) promotes innate and adaptive immunity to Mycobacterium tuberculosis.

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Abstract

Mycobacterium tuberculosis (*Mtb*) is increasingly resistant to antibiotics. Therefore, it is paramount to develop host-directed therapies (HDT) aimed at activating the host immune response to promote bacterial killing. *Mtb* is phagocytosed by alveolar macrophages (AM) and infiltrating monocyte-derived macrophages (MDM) in the lung which upregulate effector functions by epigenetic modifications to enable transcription. Suberanilohydroxamic acid (Vorinostat; SAHA), an FDA-approved histone deacetylase inhibitor, can modulate these changes and support immunity.

Human AM were purified from bronchoalveolar lavage. MDM were obtained from blood of healthy donors and patients with TB. Macrophages were infected with *Mtb* in the presence of SAHA. After 24 hours, cytokine secretion was quantified. Macrophages were washed and co-cultured with CFSE-labelled PBMC from IGRA positive donors. T-cell responses were analysed by flow cytometry and ELISA. Macrophages were lysed and colony forming units were enumerated.

SAHA increased IL-1β and decreased IL-10 in human AM and MDM infected with *Mtb*. Proliferating T-helper cells co-cultured with *Mtb*-infected, SAHA-treated AM or MDM exhibited enhanced IFN-γ and GMCSF co-production. SAHA promotes proinflammatory immune responses to Mtb infection in human AM and MDM, with a subsequent effect on T cell responses. SAHA may therefore be beneficial as a host-directed therapy or vaccine adjunct against TB.

P22

Development of a microbially-derived therapy against *Fusobacterium nucleatum*, a bacterial pathogen linked with colorectal cancer

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Abstract

Background. Specific bacterial species have been linked to several intestinal diseases, including colorectal cancer (CRC). In recent years, high abundances of an emerging pathogen, *Fusobacterium nucleatum*, have been identified in tumors and stool samples of CRC patients and it has been suggested that *F. nucleatum* contributes to CRC initiation and development. The possibility of suppressing the growth of *F. nucleatum* in the GI tract using antimicrobial-producing probiotic bacteria may reduce the overall risk of CRC development.

Methods. Here, we screen a collection of faecal samples from healthy donors against *F. nucleatum* in an effort to discover an antimicrobial-producing isolate capable of selectively inhibiting this emerging human pathogen. Potential isolates with anti-Fusobacterial activity were then further analysed for the ability to inhibit the pathogen in cell culture and in a faecal fermentation system, which simulates the dynamic conditions of the human colon.

Results. Culture-based screening of over 16,000 colonies of gastrointestinal origin resulted in the identification of one faecal isolate with probiotic potential displaying significant antagonistic activity against *F. nucleatum* initially in cell culture media and subsequently inhibition was confirmed in the simulated intestinal model.

Conclusion. This study reveals that, a novel gut isolate demonstrates inhibition against the CRC-associated *F. nucleatum in vitro* and suppresses its growth in a model of the human distal colon. This is an important finding, suggesting the potential of a natural gut bacterium to supress the growth of a bacterial pathogen associated with CRC, which may contribute to reducing the overall risk of developing the disease.

P23

Quorum quenching activity of Aerva lanata against Catheter Associated Urinary Tract Infections (CAUTI) Pathogens

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Abstract

Background: CAUTI is the most common hospital infection caused by Gram-negative bacterium owing to their ability to colonize UT mucosa. These uro-pathogens elaborate quorum sensing (QS) molecules that modify the expression and secretion of biofilm matrix compounds in response to rheological and other UT changes during catheterization. Quenching the biological effects of QS molecules will have substantial clinical value. This study identifies and evaluate the antibiofilm/anti-virulence properties of Aerva lanata plant extract against select CAUTI pathogens.

Methods: Methanolic extract of Aerva lanata was screened for its anti-urobacterial effects using *Chromobacterium violaceum* as the test strain. A. lanata extract impact on the QS molecule, acyl homoserine lactone (AHL) regulated bacterial functions essential for biofilm formation was investigated. It includes RT-PCR analysis of QS genes in the bacterium and in silico studies of bacterial compounds.

Results: Methanolic extract of A. lanata interfered with AHL regulated physiological functions such as biofilm formation, pellicle inhibition, flagellar motility and exopolysaccharides (EPS) production. In situ-visualization of biofilm with confocal microscopy showed concentration influenced reduction in bacterial biofilm formation in response to plant extract. GC-MS identified 11 compounds and molecular docking analysis predicted their respective host receptors. In silico analysis of plant extract revealed putative compounds with potential to inhibit the bacterial QS AHL system. RT-PCR analysis revealed down regulation of QS related virulence genes viz*., las1*, *lasR*, *lasB*, lasA.

Conclusion: Data obtained in this study strongly suggests that Aerva lanata should be further investigated for its therapeutic potentials to treat bacterial CAUTI.

P25

Salmonella antibiotic persistence and macrophage polarisation

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Abstract

Salmonella enterica infections have a high incidence of relapse due in part to the high levels of antibiotic persistence. It is known that growing and non-growing (*e.g.* persister) *Salmonella* utilise the *Salmonella* Pathogenicity Island 2 (SPI-2) Type 3 Secretion System (T3SS) to dampen the pro-inflammatory M1 state of macrophages and push the immune cells towards an anti-inflammatory M2 state. This allows *Salmonella* prolonged survival in the host and facilitates relapse. However, this interplay between *Salmonella* survival and macrophage activation states remains incompletely understood.

Here, I present a study on the influence of the initial phenotypic state of untreated (M0), IFN-γ (M1) and IL-4/IL-10 (M2) prepolarised differentiated murine bone marrow-derived macrophages (MΦs) on the survival and proliferation of *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). As expected, pro-inflammatory M1 MΦs kill *S*. Typhimurium more efficiently than M0 cells. Surprisingly, *S.* Typhimurium shows decreased survival within anti-inflammatory M2 MΦs compared to M0 MΦs, which appears to be SPI-2 dependent. Further investigation revealed that even though *S.* Typhimurium induces SPI-2 T3SS within M1 and M2 prepolarised MΦs, the timing of SPI-2 activation is affected during infection of M2 MΦs, thereby reducing the ability of bacteria to proliferate. This leads to many more antibiotic tolerant non-growing *S.* Typhimurium surviving to combined antibiotic and macrophage challenges in prepolarised MΦs.

Together, these results suggest that infection of M0 MΦs followed by reprogramming to M2 MΦs appears to promote the optimal conditions for *S*. Typhimurium proliferation; nevertheless, non-growing cells may immediately persist and survive better in prepolarised MΦs.

P26

Whole genome sequencing reveals genetic diversity in Mycobacterium avium subspecies paratuberculosis population circulating in Irish cattle.

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's Disease (JD), a chronic enteritis, in cattle. Whole genome sequencing (WGS) has been applied to many pathogen systems, where its unprecedented resolution has greatly enhanced our understanding of the molecular epidemiology of pathogen transmission. However, WGS has seen limited application to MAP; understanding the transmission dynamics of MAP will inform control of JD. We report the first study into the application of WGS to MAP in Ireland.

DNA was extracted from 167 MAP isolates sourced from cattle across Ireland. Libraries were prepared and sequenced on an Illumina-NextSeq500 platform. Sequencing data were processed using an in-house bioinformatic pipeline, which trimmed reads, aligned them to the reference genome MAP K10, followed by variant calling, quality filtering and construction of a maximum-likelihood phylogeny. DNA extracts were also used for MIRU-VNTR typing.

The resulting phylogeny shows that the MAP population present in Irish cattle is genetically diverse, which may have resulted from importation of MAP strains from across Europe into Ireland. Similar diversity was observed in a WGS study that noted the impact of cattle imports on the Canadian MAP population. Some Irish isolates were genetically similar to European and Canadian isolates.

Comparing our WGS data with MIRU-VNTR indicates that MIRU-VNTR has limited resolution for discriminating MAP strains, and often does not distinguish isolates to sufficient resolution.

The genomic data presented here provide the first snapshot of genetic diversity of Irish MAP and a baseline for future studies into spread and persistence of MAP in Irish cattle.

P27

Use of genomics to study antimicrobial resistance in *Mycobacterium avium* subsp. *paratuberculosis*

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Abstract

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. The rise in antibiotic use in both the agricultural livestock industry and in human healthcare has led to the emergence of antimicrobial resistance (AMR). This now presents a global threat to public health. Isolates from clinical samples have been seen to be resistant to most drugs among them, serious human pathogens including *Mycobacterium tuberculosis, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Neisseria gonorrhoeae, Acinetobacter baumannii* and *Pseudomonas aeruginosa.* Microbes have the ability to harbour and exchange antimicrobial resistance genes.

Understanding the differences between different strains of *Mycobacterium avium* subsp. *paratuberculosis (MAP)* is important in knowing how they influence both the development and transmission of diseases. Microbial whole genome sequencing (WGS) has the potential to accelerate the detection of AMR in pathogens. Using these technologies and available databases, we were able to elucidate antimicrobial resistance mechanisms, antimicrobial resistance genes and antimicrobial classes. Here we examine the presence of AMR genes in over 200 strains of MAP. The objective of this study was to examine the use of bioinformatics tools to predict AMR in strains of MAP.

P28

Lactate improves killing of Mycobacterium tuberculosis in human macrophages

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Abstract

Mycobaterium tuberculosis (Mtb) kills more people than any other infectious agent worldwide. Our group has shown the importance of glycolysis in controlling intracellular Mtb growth in human macrophages[1], which leads to increased lactate production. Therefore, we examined if lactate had an immunomodulatory effect on human monocyte derived macrophages (hMDM) infected with Mtb.

hMDM were adherence purified from healthy donor buffy coats with human serum for 6-8 days. Sodium L-Lactate was added 3 hours prior to infection with Mtb or stimulation with LPS (100 ng/ml). Macrophage phenotype and function was assessed by ELISA, flow cytometry and Seahorse metabolic-flux analysis. Bacillary killing was determined by colony forming units (CFU) after lysing infected hMDM, plating on middlebrook agar and counting 21 days later.

25 mM of lactate improves hMDM ability to control intracellular Mtb growth with a 55% reduction in CFU at day 5 post infection. The glycolytic response seen immediately following infection with Mtb or LPS stimulation was decreased with lactate. LPS-induced upregulation of activation markers was diminished by lactate.

Lactate is a product of aerobic glycolysis induced by infection and supports the intracellular killing of Mtb. Further research is required to elucidate the mechanism of this effect.

1. Gleeson, L. E. *et al.* Cutting Edge: Mycobacterium tuberculosis Induces Aerobic Glycolysis in Human Alveolar Macrophages That Is Required for Control of Intracellular Bacillary Replication. *J. Immunol.* **196**, 2444–2449 (2016).

P29

Desferrioxamine supports innate immune function in human macrophages infected with *Mycobacterium tuberculosis*

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Abstract

Antimicrobial resistance in tuberculosis poses a major global health challenge. The development of host-directed therapies (HDTs) which can be used in combination with existing antimicrobials, are urgently required. Our research focuses on strategies aimed at promoting immune cell function, early killing and removal of Mycobacterium tuberculosis (Mtb) from macrophages following infection. HIF1α-mediated induction of aerobic glycolysis is integral to the host macrophage response during infection with Mtb, as this promotes bacillary clearance. Iron chelators have been shown to modulate cellular metabolism through the regulation of HIF1α. We examined if the iron chelator, desferrioxamine (DFX), could support the function of primary human monocyte-derived macrophages and human alveolar macrophages infected with Mtb, as previously hypothesized (1). We demonstrate that DFX supports the production of cytokines integral to macrophage immune function and enhances aerobic glycolysis in these macrophages. Moreover, we show that DFX primarily modulates the function of these cells in a HIF1α-dependent manner. Preliminary evidence also suggests that DFX exhibits additional effects during Mtb infection by enhancing the bactericidal efficacy of antimicrobials and by recruiting other innate immune cells to help clear the infection. Collectively, our data suggests that DFX exhibits potential as a HDT to enhance immunometabolic responses, boost host immune function and aids in early bacterial clearance during Mtb infection (Funding: The Royal City of Dublin Hospital Trust and Science Foundation Ireland).

Reference(s)

1. Phelan JJ, Basdeo SA, Tazoll SC, McGivern S, Saborido JR, Keane J. Modulating Iron for Metabolic Support of TB Host Defense. Frontiers in immunology. 2018;9:2296.

P30

Polysaccharide-Dependent Biofilm Formation is induced by Bile in a Cystic Fibrosis Isolate of *Staphylococcus aureus*.

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Abstract

S. aureus is the pathogen most frequently isolated from cystic fibrosis (CF) patient airways, particularly children and adolescents. Fundamental knowledge of the molecular pathogenesis of *S. aureus* infection in the CF lung is lacking. A large percentage of CF patients suffer from gastroesophageal reflux resulting in bile aspirating into the lungs. Bile exposure likely affects pathogen physiology and was previously found to enhance biofilm formation by some *S. aureus* strains. This study aims to investigate the effect of bile on *S. aureus* biofilm formation.

Two *S. aureus* isolates of the same strain obtained from a CF patient's sputum, 26 months apart, were tested for the ability to form biofilm. While the initial isolate (CF0) formed biofilm in NaCl-supplemented media, the isolate recovered 26 months later (CF26) did not. CF26 biofilm formation was induced by addition of bile to NaCl-supplemented media.

S. aureus biofilms are mediated by the *icaADBC*-encoded polysaccharide intercellular adhesion (PIA) or surface proteins. Proteinase K did not affect biofilm density while sodium metaperiodate degraded bile-induced CF26 biofilm indicating polysaccharide rather than protein is essential to biofilm integrity and that CF26 forms PIA-dependent biofilm. To examine if bile alters expression of the *icaADBC* operon, real-time PCR was carried out. Similar levels of *icaA* transcript was measured in CF26 grown with and without bile indicating that the failure of CF26 to form biofilm in the absence of bile is not due to a failure to express the operon. We hypothesise that there may be reduced PIA export or cell surface anchorage.

P31

The effect of teat sealant alone at dry off on subsequent SCC and infection rates in dairy cows and heifers

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Abstract

The dry period is of critical importance for mastitis control, 1) to cure existing infections and 2) to prevent new infections. This is achieved through blanket antibiotic treatment and teat sealant (AB+TS) at dry off. This trial investigated the effect of 1) administering teat sealant (TS) only at dry-off 2) the effect of administering TS to heifers in the high risk dry period, on somatic cell count (SCC) and bacterial presence in the following lactation. Cows with SCC of <200,000 cells/mL were randomly assigned TS or AB+TS, while heifers were administered TS in contralateral quarters (front right and left hind). The remaining quarters acted as controls. Cow, quarter SCC and bacteriology data was collected. Cows on AB+TS had a significantly ($p<0.05$) lower mean SCC (60,483cells/mL) over the lactation compared to TS cows (80,900cells/mL). At 240+ days in milk, the proportion of cows with SCC >200,000cells/mL was similar for both treatments (30%). Bacteria were present in 6% of the quarters treated with TS while 2.6% treated with AB+TS. Heifer teats not administered TS were more likely to have bacteria present: 3.85 times ($p<0.001$) at calving and 1.99 times ($p<0.05$) at mid lactation. Teat sealant had no effect on SCC of heifers at calving ($p=0.52$) or mid lactation ($p=0.35$). Herds with effective mastitis control and low SCC can use TS alone at dry off on cows with low SCC with minimal effect on SCC and infection rates. Teat sealant in heifers reduces infection rates in herds with problematic clinical mastitis.

P32

Using metagenomics to investigate the impact of hospital stay and the ARK intervention on the human gut resistome

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Abstract

Antimicrobials are vital for modern medicine. Antimicrobial use selects for antimicrobial resistant bacteria, particularly among the gut microflora. Minimising antimicrobial resistance (AMR) selection by avoiding unnecessary antibiotic use helps combat AMR. Metagenomic analyses have the potential to provide accurate detection and quantification of AMR genes within an individual's gut microbiome (gut 'resistome'), allowing the impact of different types of antibiotic exposures to be evaluated and guide interventions to reduce AMR.

We have developed a short-read sequencing approach to characterise the gut 'resistome' and piloted this in two clinical sample sets.

The first consisted of paired stool samples from older hospitalised adults. 25 pairs of samples (1 to 50 days apart) showed a median AMR gene reads/kb/million total reads (RPKM) of 1841 (124 to 17,832), and a median AMR gene count of 55 (2 to 101).

The second consisted of faecal discards from *Clostridium difficile* testing at a hospital eleven months apart. In these samples (n=21) the median AMR gene read was 923 RPKM (240 to 19,475), and the median AMR gene count was 36 (9 to 82).

In both sample sets there was a trend towards an increase in AMR gene RPKM and number between the time points. dfrF, ErmB and tetW were the commonest AMR genes in both sample sets.

This approach is being applied to analyse the impact of an intervention (ARK-Hospital) designed to change antibiotic prescribing behaviour. The data will allow us to determine the patient-level impact of reduced antibiotic exposure on carried AMR.

P33

The molecular basis of antibiotic treatment failure in chronic urinary tract infections

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Abstract

Urinary tract infections (UTIs) are amongst the most common infections worldwide, and are becoming increasingly difficult to treat. In addition to the acceleration of classic antimicrobial resistance, recurrence after initial resolution is common. Our clinical experience is that chronically infected patients sometimes fail to respond to antibiotics predicted to be effective from culture-based sensitivity testing, while antibiotics predicted to be unsuitable can succeed. We hypothesized that the bladder environment could lead to differential bacterial gene expression, resulting in differences in minimum inhibitory concentration (MICs) compared with standard culture.

Here, using strains of *Escherichia coli* evolved in the lab to be resistant to amoxicillin–clavulanic acid, we present data that MICs differ depending on which media the assay is performed in (M9, ISO, LB, human urine), as well as in urine-containing supernatant enriched from urothelial organoids. Next, we examined the behaviour of patient-derived *Enterococcus faecalis*, one of the main causative agents of chronic UTIs in the elderly. We are in the process of evaluating the MIC of first-line UTI antibiotics using growth media supplemented with urine, to more closely mimic the native uropathogen environment. Moreover, we are characterising the resistance genes expressed in those differing environments using next generation sequencing technology and comparing the results with those obtained from bacteria grown on standard diagnostic media.

Our work demonstrates the danger of extrapolating biological behaviour from artificial culture substrates and may lead to better diagnostic tests and treatments for chronic UTI.

P34

Socially messaging polycellular interactomes for innovative interventions in health and medicine

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Abstract

The fast approaching post-antibiotic era has focused efforts on the search for new classes of antimicrobial compounds to arrest the increase in mortality levels attributed to microbial infections. Key to this has been the realisation that persistent infections arise from changes in polymicrobial communities, and the interactions between the protagonists within these communities can markedly affect the effectiveness of antibiotic-based interventions. Understanding the impact of key interactions within these ecosystems is key to priming the effectiveness of drug-based interventions.

We are pursuing a chemico-biological approach to investigate the molecular mechanisms underlying the anti-infective activity of small molecules from microbe-host interactomes. Evolved through co-existence, these novel molecular entities offer an effective means of pathogen control through a form of ‘chemical messaging’. Accessing the thesaurus of microbial messaging through rational design has led to a suite of distinct bioactive frameworks that can influence inter-species behaviours, moderating chronic and acute phases of virulence and pathogenesis in *Pseudomonas aeruginosa* and other pathogens. Similarly, at the interkingdom level, small molecular entities with anti-inflammatory and anti-cancer activities have been identified. Elucidating the molecular mechanisms underpinning the bioactive potential of these small molecules is key to unlocking their potential to deliver next generation medicines. This can only be done with a deeper understanding of the pathoadaptive phenotypic and genotypic heterogeneity that is emerging from clinical studies.

P35

Mutation of succinyl-CoA synthetase in the tricarboxylic acid cycle decreases β-lactam resistance in MRSA

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Abstract

Antimicrobial resistance (AMR) is a worldwide public health issue that poses a serious threat to the effective treatment of infectious diseases. *Staphylococcus aureus* is one of the most frequent causes of antibiotic-resistant infections, prevalent in both the healthcare and community settings. Its ability to adapt and continually acquire resistance to antimicrobials makes it a major concern in modern health care. A phenotypic screen of the MRSA Nebraska Transposon Mutant Library identified succinyl-CoA synthetase mutants NE569 (SucC; β-subunit) and NE1770 (SucD; α-subunit) that are clinically susceptible to β-lactam antibiotics and produce small, non-pigmented colonies. This loss of resistance is independent of *mecA* expression and is reproducible in additional MRSA backgrounds. Proteomics analysis is currently underway to investigate if an accumulation of the SucCD substrate, succinyl-CoA, influences β-lactam resistance via altered post translational protein succinylation. Revertants of NE569 and NE1770 in which b-lactam resistance and wild type colony morphology was restored readily emerged in the absence of antibiotic pressure without loss of the *sucC* or *sucD* transposon insertions. Whole genome sequence analysis of selected reverants is underway to identify the mutation(s) responsible. This work encapsulates the broader challenge faced in medical microbiology of identifying potential therapeutic targets while recognising the immense potential of *S. aureus* to respond to such advances.

P36

Mutations in the sigB operon of Listeria monocytogenes confer a growth and competitive advantage at higher temperatures

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Abstract

Background: The food-borne pathogen *Listeria monocytogenes* is a highly stress resistant bacterium. Such resistance is partly determined by the activation of the alternative sigma factor B (σ^B). Genes within *sigB* operon encode regulatory proteins that transduce stress signals leading to the activation of σ^B.

Objectives: The goal was to characterize the impact of secondary mutations that arose in the *sigB* operon during the construction of a transposon mutant library and to identify the conditions that promote the selection of such mutations among wild-type populations.

Methods: Whole genome sequencing was performed in *L. monocytogenes* transposon mutants that had lost their characteristic phenotype associated with σ^B activity. Acid tolerance (pH 2.5) and σ^B activity was measured in these strains. Growth kinetics was measured at different temperatures. Competition experiments were performed using mixed cultures incubated at 42°C.

Results: Frameshift mutations within the *sigB* operon were identified in *rsbS*, *rsbU* and *rsbV*, which showed decreased acid tolerance and reduced in σ^B activity. Increased growth rates of the *sigB* operon mutants and Δ*sigB* was observed when compared to the WT during the transition to stationary phase at elevated temperatures (40-42°C). The *sigB* operon mutants and Δ*sigB* were able to overtake the WT population in mixed culture competition experiments. Elevated growth temperature can result in the selection for mutations that result in deceased σ^B activity in *L. monocytogenes*. We speculate that the growth advantage arises in sub-lethal conditions due to improved resource allocation (energy and transcriptional machinery) towards growth in mutants that cannot fully deploy the general stress response.

P37

Longitudinal *Staphylococcus aureus* nasal colonization and microbiome disruption in adolescents

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Abstract

For *Staphylococcus aureus*, colonization is the antecedent of both disease and transmission, but the determinants of colonization in human populations are not well understood. We hypothesized that resident microbiota have an impact on the ability of *S. aureus* to establish sustained colonization. To test this hypothesis we longitudinally sampled the nares of 21 healthy adolescents over 5 non-consecutive weeks during February and March 2015. We subjected each sample (n=89) to culture-independent microbiome (16S rRNA gene-based) analysis, as well as culture and whole genome sequencing of *S. aureus* isolates. *S. aureus* was isolated from 46% of samples. Nine participants (42%) were persistently colonized, 4 (19%) were intermittently colonized, and 8 (38%) had no *S. aureus* on the days tested. Whole genome analysis of all 43 distinct *S. aureus* isolates showed a wide diversity of strains belonging to 9 distinct clonal complexes, and phylogenetic analysis showed that all persistent carriers and some intermittent carriers harbored a single strain over time. The presence of *S. aureus* was associated with specific differences in the nasal microbiota including a positive correlation between *S. aureus* colonization and abundance of *Streptococcus* ($p=0.0004$) and *Neisseria* ($p=0.0056$), and a strong negative correlation with *Corynebacterium* abundance ($p<0.0001$). Culture-independent results showed that abundance of the genus *Staphylococcus* correlated with the genera *Streptococcus* and *Brevibacillus* ($p=0.0004$ and 0.0021, respectively), and was inversely correlated with *Moraxella* abundance ($p<0.0001$). Although the nasal microbiota remained relatively stable over time, with higher intersubject than intrasubject beta diversity ($p<0.0001$), microbiome profiles from individuals with *S. aureus* were significantly more variable over time ($p<0.0003$). These results suggest that colonization with *S. aureus* is associated with specific differences in the diversity of the nasal microbiota. We suggest that microbiome stability may be a critical factor in determining the ability of *S. aureus* to colonize and persist.

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