ECM Forum Summer Conference 2018
Poster Abstract Book

Contents

Posters for the ECM Forum Summer Conference will be presented from 17:30 – 19:00 on Thursday 14 June.

Contents (continued)

P1

Does lipid remodelling play a key role in host-pathogen interaction and antimicrobial resistance in critical priority pathogen *Pseudomonas aeruginosa*?

Rebekah Jones, Yin Chen

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Abstract

Pseudomonas aeruginosa is a significant nosocomial pathogen, alongside prevalence in immunocompromised individuals, with multi-drug resistance capabilities. *P. aeruginosa* is able to live in complex environments, owing to a large genome, suggesting readiness to adapt. Lipid remodelling is the process by which bacteria selectively modify their membrane lipid composition in response to environmental stimuli, in particular low phosphate conditions. A recently identified lipid remodelling protein in marine bacteria, an intracellular phospholipase C (PlcP), shares 47% protein sequence similarity with a putative *P. aeruginosa* PlcP. This enzyme is hypothesised to cleave membrane phospholipids, liberating diacylglycerol to be used as the base for synthesising non-phosphate containing lipids. Here, the role of the PlcP homologue in *P. aeruginosa* lipid remodelling in response to clinically relevant low phosphate conditions is investigated. Additionally, a glycosyltransferase (Agt) located in operon with PlcP is reviewed for its role in glycolipid production. High performance liquid chromatography coupled to mass spectrometry (HPLC-MS) confirmed reduction in phospholipids and increased production of non-phosphate glycolipids and ornithine lipids in *P. aeruginosa* in response to low phosphate conditions. Heterologous expression of Agt in *Escherichia coli* elicited production of glycolipid monoglycosyl diacylglycerol (MGDG). A *Caenorhabditis elegans* infection model to reveal differences in lethality between wild type *P. aeruginosa* and mutants in lipid remodelling will be used. Given that the membrane is the first to encounter host cells or antimicrobials, it is of significant interest in terms of the multi-drug resistance arsenal of *P. aeruginosa*.

P2

The development of an ex vivo, porcine, lung model of cystic fibrosis to support mixed *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilm.

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Abstract

Chronic infections of the cystic fibrosis lung are poly-microbial. Robust laboratory models of multi-species biofilm infections are therefore needed to investigate bacterial interactions and the effects of antimicrobial treatment on a complex bacterial microbiota. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are both dominant organisms associated with airway infections of cystic fibrosis patients and their interactions are considered important for the clinical outcome of disease. We have previously described an *ex vivo* pig lung model (EVPL) of cystic fibrosis that demonstrates the influence of host tissue interaction and biofilm structure on the growth and persistence of *Pseudomonas aeruginosa* lung infection. Here we show the progression of this model to support the growth of *Staphylococcus aureus* and the potential to establish mixed *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilm to further our understanding of inter-pathogen interactions in poly-microbial lung infections.

P3

Intracellular survival of *Enterobacter cloacae* complex in human macrophages may interfere with the function of pannexin-1.

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Abstract

The multidrug-resistant ESKAPE pathogens are the leading causes of nosocomial infections. *E. cloacae* (represented by the last "E"), are Gram-negative bacteria commonly isolated from nosocomial outbreaks. *E. cloacae* can infect several body systems, mainly in immunocompromised patients, ranging from the CNS to the GI tract, and many more. Unfortunately, minimal information exists on the virulence-associated properties and pathogenicity of *E. cloacae*. The aim of this study is to explore how *E. cloacae* interacts with human macrophages and identify the bacterial and host cell factors involved. This is based on our preliminary data supporting the hypothesis that *E. cloacae* specifically inhibits the function of the PANX1 channel in human macrophages, leading to the formation of membrane protrusions and cell fragmentation. Differentiated THP-1 cells and iBMDMs were infected with m-Cherry and GFP fluorescent *E. cloacae* for various lengths of time. Infection was analysed by confocal microscopy, focusing on the morphology of the infected macrophages and *E. cloacae* bacilli. Images were then analysed on ImageJ.Morphological changes could be seen in both infected iBMDMs and THP-1 macrophages. These changes resembled the morphology observed by chemically blocking the PANX1 channel in other cell types, such as Jurkat cells. As the phenotype requires live, intracellular *E. cloacae*, we speculate it is due to the secretion of bacterial effector proteins (or toxins) that remain to be elucidated, a process ongoing in our laboratory. Understanding the molecular and cellular pathogenesis of *E. cloacae* could lead to the development of newer treatment approaches as alternatives to antibiotics.

P4

Microbial community interactions: An investigation of niche-packing and coevolution in coalescence.

Meaghan Castledine, Daniel Padfield, Angus Buckling

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Abstract

Microbial community coalescence (mixing of multiple communities) is ubiquitous, from multi-species exchanges in human contact to utilisation in medicinal treatments. Interestingly, some microbial communities can form cohesive units and asymmetrically dominate others; yet the mechanisms which determine the outcomes of coalescence are not fully understood. One proposal is the niche-packing hypothesis which predicts that communities which utilise resources more efficiently will be more successful. This has had some support from studies linking increased community diversity to invasion resistance; however, this hypothesis is still to be tested from an evolutionary perspective in coalescence. As coevolution shapes the way in which microbial communities exploit resources, by selecting for species to reduce niche overlap (i.e. adaptive radiation, character displacement), we hypothesise that coevolution increases community cohesion. We tested this using the bacterium *Pseudomonas fluorescens* which diversifies into communities of niche specialist morphotypes. Here, we measured relative community success and morphotype fitness in competition trials, simultaneously testing the role of coevolution and diversity in community coalescence and invasion. Community coexistence occurred, irrespective of differences in intra-community coevolution between coalesced communities which suggests niche-partitioning evolved in parallel. Whilst diversity increased community success, this effect was driven by morphotypes being in the presence of a community member of the same genetic background. Therefore, this suggests coevolution may be important for individual relative fitness against invaders. Hence, this study highlights mechanisms shaping interactions of microbes and their communities: principles essential for understanding the role of microbiomes in health and disease.

P5

Microbiological analysis of flying insects collected in the hospital environment and antibiotic resistance profiles of isolated bacterial strains.

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Abstract

Insects are efficient vectors of bacteria and in the hospital environment they likely play a role in spreading nosocomial infections. This study examines the microbial community of flying insect populations collected from seven UK hospitals, focusing on the antibiotic-resistance profile of bacterial isolates. Insects were collected with ultra-violet (UV) light flytraps and professional sticky traps. Bacteria carried on the insects were isolated using traditional microbiological culture techniques and the isolates identified using macroscopic morphology, Gram staining, biochemical properties and metabolism. Kirby – Bauer disc diffusion method was performed to assess the antibiotic-resistance profiles of the bacterial isolates. From 19,937 individual insects collected in the study, 79 bacterial strains were isolated. The majority of bacteria belonged to Enterobacteriaceae Family (42 %), followed by *Bacillus* spp. (24 %) and *Staphylococcus* spp. (19 %). Less predominant were bacteria from Genus Clostridium (6 %), *Streptococcus* spp. (5 %) and *Micrococcus* spp. (2 %). Of a total of 68 strains tested for their antibiotic resistance profile, 52.9 % showed a resistant phenotype to at least one class of antibiotics. *Staphylococcus* spp. showed the highest percentage of resistant strains (83.3 %), followed by *Bacillus* spp. (60 %) and Enterobacteriaceae (31.3 %). These findings show that a variety of flying insects collected in the hospital environment carried potentially pathogenic bacteria with some antibiotic resistance. Insects have a potential role in spreading antibiotic-resistant strains and, therefore, could increase human exposure to drug-resistant bacteria. This study highlights the importance of pest control as part of infection control strategies in hospitals and healthcare facilities.

P6

The pangenome of *Aspergillus fumigatus*.

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Abstract

The concept of the species "pangenome", the union of all "core" conserved genes and all "accessory" non-conserved genes across all strains of a species, was first developed in prokaryotes to account for intraspecific variability. The concept has recently been extended to eukaryote genomics and evidence for eukaryotic pangenomes has been demonstrated in plants, plankton and fungi. Using a previously published method based on sequence homology and conserved microsynteny in addition to bespoke processing pipelines, we have investigated the pangenomes of four exemplar fungal species (*Saccharomyces cerevisiae*, *Candida albicans*, *Cryptococcus neoformans* var. *grubii*, *Aspergillus fumigatus*). Detailed analysis of the pangenome of *Aspergillus fumigatus* using 12 strain genomes reveals that on average 83% of genes in a given *A. fumigatus* strain's genome are syntenically-conserved core genes. This core *A. fumigatus* genome is enriched for many different housekeeping processes. The remaining 17% of accessory genes include many paralogs of both core and other accessory genes as well as horizontally-transferred genes, and are enriched for extracellular transport and protein modification processes. Mapping of the core and accessory complements of the *A. fumigatus* pangenome onto the genome of the reference Af293 strain shows "islands" of variable gene content within most of the eight *A. fumigatus* chromosomes, particularly in subtelomeric regions. Overall there is strong evidence for pangenomic structure in fungal species, and in *A. fumigatus* gene duplication appears to have some influence on the development of the species pangenome.

P7

Exploring the Role of VpsT in *Vibrio cholerae* Lifestyle Switching.

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Abstract

Vibrio cholerae is an aquatic bacterium and the cause of cholera which remains a significant cause of endemic and epidemic disease particularly in areas where local infrastructure has been damaged and/or there is limited access to clean water.

Between outbreaks *V. cholerae* can persist in aquatic environments by colonising alternative hosts (such as fish) and by forming biofilms on chitinous surfaces. Vibrio polysaccharide (VPS) is necessary for the formation of biofilms and its production is co-ordinated by a complex regulatory network that links quorum sensing with the bacterial second messenger cyclic-di-GMP. C-di-GMP is known to up-regulate the expression of genes involved in biofilm formation in several bacterial pathogens.

In *V. cholera* c-di-GMP activates VpsT, a transcription factor that is known to regulate the expression of *vps* genes.

To better understand the global role of VpsT, we used ChIP-seq to investigate its binding distribution across the genome of *V. cholerae*. Our data identifies additional, uncharacterised VpsT targets, possibly expanding the VpsT regulon. These targets include genes encoding proteins involved in motility and adhesion, as well as the degradation of c-di-GMP.

P8

In vitro* Antimicrobial Activity of *Cymbopogon flexuosus* Against *Staphylococcus aureus* and *Acinetobacter baumannii.

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Abstract

Staphylococcus aureus (*S. aureus*) and *Acinetobacter baumannii* (*A. baumannii*) are both significant nosocomial pathogens that have become increasingly resistant to conventional antimicrobial therapies. There is a need for alternative therapies to combat this issue and recent literature has shown that essential oils can be effective.

This study investigated the antimicrobial effect of *Cymbopogon flexuosus* (lemongrass) against the mentioned pathogens, using the disc diffusion assay and broth microdilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as well as growth kinetics using the optical density method.

The results of the disc diffusion assays showed that lemongrass was effective against both organisms, though less so against *A. baumannii,* likely due to the intrinsically resistant nature of Gram-negative bacteria. The broth microdilution method showed the low concentrations of lemongrass could inhibit and kill each organism, 0.06% (v/v) and 0.13% (v/v) for *S. aureus* and 0.13% (v/v) and 0.25% (v/v) for *A. baumannii* respectively. These findings were corroborated by the 96-hour optical density growth kinetics, which identified the same MIC and MBC and the broth microdilution method. This suggests that the optical density growth kinetic method of determining MIC and MBC could, if not replace, the corroborate results from the more commonly used methods.

P9

Isolation and characterisation of bacteriophages for use against biofilms of *Pseudomonas aeruginosa* clinical isolates.

Joshua Box, Dann Turner, Shona Nelson

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Abstract

Pseudomonas aeruginosa is a World Health Organization priority 1, ESKAPE pathogen that is capable of causing a wide spectrum of nosocomial and community-acquired infections. It employs a wide range of antibiotic resistance mechanisms and is often difficult to treat. It is a potent biofilm former and is often associated with infections on medical devices and in cystic fibrosis. Biofilms are a commonly employed mechanism by bacteria to aid survival and it is thought that most infections are associated with biofilm formation. Given the rise of antimicrobial resistance and the paucity of new antibiotics, novel antimicrobial therapies are required to combat resistant bacteria. In this study, environmental bacteriophages were isolated against a panel of clinical isolates of *P. aeruginosa*. A total of 15 potentially unique bacteriophages were isolated from activated sludge obtained from Avonmouth Sewage Treatment Works, Bristol. These bacteriophages were propagated and host ranges established against a panel of 10 unique clinical strains of *P. aeruginosa*. Of these, 3 exhibited host range of above 50% and were further characterised by transmission electron microscopy. Bacteriophages vB_PaeM-SM152, vB_PaeM-SM331 and vB_PaeM-SM332 are all members of the family *Myoviridae* with an average tail length of 185±13.79 nm, 193±0.22 nm and 196±7.31 nm and isometric capsid diameter of 119±1.17 nm, 123±8.92 nm and 121±2.55 nm, respectively. This characterisation informed which of the isolated bacteriophages were used against biofilms formed from clinical isolates of *P. aeruginosa*.

P10

Physiological characterisation of a new *Salmonella* Enteritidis variant that is responsible for blood stream infection in Africa.

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Abstract

An epidemiological paradox has begun to emerge regarding the outcome of non-Typhoidal *Salmonella* (NTS) infection in high- and low- income settings. In high-income settings, NTS infection causes self-limiting gastroenteritis with very few deaths. In Africa, however, poor antibiotic stewardship and the high prevalence of immunosuppressive disease has led to NTS emerging as a leading cause of invasive disease (iNTS), characterised by bloodstream infection, in both adults and children (Ao *et al.*, 2015, *Emerg. Infect. Dis*.).

The African iNTS epidemic is caused by *Salmonella enterica* serovars Typhimurium and Enteritidis. Much effort has focused on *S*. Typhimurium and the factors controlling the invasive lifestyle of the pathogen. However, limited attention has been paid to *S.* Enteritidis. The lack of research into invasive *S.* Enteritidis has led to a gap in our knowledge regarding the African iNTS epidemic. My work aims to reduce this gap by conducting an extensive physiological characterisation of over a 100 African *S.* Enteritidis strains.

Previous work by Feasey *et al*., (2016, *Nature Genet*.) identified three distinct clades involved with the African S. Enteritidis epidemic; the global epidemic clade (associated with enteritis), Central and East African clade (associated with iNTS) and West African clade (associated with iNTS). I will present new data that identify distinct phenotypes associated with the African clades of *S.* Enteritidis, including biofilm formation and the ability to catabolise certain carbon sources.

P11

Characterisation of a dual species perfusion biofilm model.

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Abstract

An estimated 65–80% of human infections are biofilm-related. As the biofilm mode of growth significantly contributes to bacterial tolerance of antimicrobial treatment, this has important ramifications for patient morbidity and associated healthcare costs. Biofilm infections are commonly established by more than one bacterial species which demonstrate synergistic interactions and altered behaviour, presenting yet more challenges to effective treatment. The aim of this study was to characterise a new continuous perfusion model for the culture of dual species biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and to compare the antibiotic sensitivities of cells eluted from the system with biofilm cells.

Biofilms of either *S. aureus* or *P. aeruginosa* were established within foam plugs, continually perfused with nutrient broth and challenged with the other species after 24 h. Biofilms were disaggregated after a further 24 h and relative species numbers determined by viable counting. Antibiotic susceptibilities of disaggregated biofilm and eluted cells were assessed by disc diffusion.

P. aeruginosa predominated in dual species biofilms, reaching 2 x 10^9 cfu/biofilm after 48 h. The *S. aureus* population was consistently lower, even when established before *P. aeruginosa* challenge (4.47 x 10^8 cfu/biofilm at 48 h and 5.5 x 10^8 cfu/biofilm 24 h post-challenge). Antibiotic susceptibility testing demonstrated no significant difference between eluted and disaggregated *P. aeruginosa* biofilm cells. *S. aureus* small colony variants (SCVs) were isolated from challenged biofilms, a likely result of co-culture with *P. aeruginosa*, and of importance due to their resistant phenotype.

P12

Do biofilms form on lead pipes?

Kim Patel, Paul Humphreys, Jeremy Hopwood

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Abstract

Lead was used in domestic water pipes for many years in the UK and despite replacement programmes operated by the water authorities there are still many households with lead in their supply chain. Lead concentrations in domestic supplies are controlled through the addition of phosphates which cause the formation of low solubility lead phosphate minerals. Whilst the mineralogy of this process has been investigated there is little investigation of the microbial component of this process. By utilising the low levels of organic carbon within the water system biofilms are able to form on the internal surface of the pipes. Here we present the first characterisation of these biofilms through optical, Confocal Laser Scanning Microscopy (CLSM), Scanning Electron Microscopy (SEM) and bacterial community analysis (pending).

P13

Improving the Diagnosis of Epizootic Lymphangitis in Working Equids Using Real-time PCR.

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Abstract

Epizootic lymphangitis (EZL) is a form of mycosis caused by the dimorphic fungal pathogen *Histoplasma capsulatum var farciminosum* (HCF). EZL is a highly prevalent disease in cart-horses in Ethiopia with occasional reports in other countries located in sub-Saharan Africa and parts of Asia. Cart-horses in subsistence economies are heavily relied upon for transportation, trade and agriculture. The morbidity of a horse can result in a significant loss of earnings in poorer regions of the world where resources are already limited. There are 4 clinical presentations of EZL, one being asymptomatic, this can allow carriers to remain undetected and undiagnosed. This in conjunction with sporadic reporting of clinical cases, dated epidemiological studies and poor diagnostic capabilities leaves an unclear picture of this potentially fatal disease. The current gold standard for the diagnosis of EZL is microbial culture. HCF culture can take between 6-12 weeks to confirm the diagnosis. Molecular diagnostic methods such as real-time PCR offer a rapid and sensitive diagnostic platform. This study aims to validate, for the first time, a real-time PCR assay to detect HCF in an array of clinical and environmental samples. Using recombinant plasmid standards to optimise the first real-time PCR assay to quantify HCF. This quantitative approach could reveal environmental vectors for disease carriage, identify the pathogenic load of HCF in typical clinical cases and potentially asymptomatic carriers within equine populations. These data could provide a much needed foundation to understand the epidemiology of disease and to inform the direction of disease intervention strategies.

P14

Interference of the ADP ribosyltransferase gene in *M. smegmatis* using the CRISPRi/dcas9 interference system.

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Abstract

Mycobacterium tuberculosis (Mtb), the causative agent of human tuberculosis, is a major global public health burden. The disease can persist for decades as an asymptomatic latent infection and mechanisms underlying persistence are poorly understood. Long treatment regimens and the emergence of multi-drug resistance have hindered control and eradication programs, hence the development of effective new treatments is necessary. Rifampicin (Rif) is a first line drug used in *Mtb* treatment, but the emergence of Rif resistant strains has had a major effect on prognosis and treatment strategies. *M. smegmatis*, a fast-growing mycobacterium commonly used as a model for *Mtb* research carries the ADP ribosyltransferase (*arr)* gene which inactivates Rif in this strain. Previous studies have shown that deleting the *arr* gene sensitises the bacteria to Rif. We have used the Clustered Regularly-interspaced Short Palindromic Repeat interference dcas9 system, (CRISPRi/dcas9) to inhibit the expression of *arr* in a dose dependent manner using the inducible expression of an enzymatically inactive cas9 protein (dcas9) and *arr* gene specific guides. Inducible inhibition of *arr* gene expression has been observed using REMA assays and RTq-PCR, resulting in up to a 6 fold sensitization to Rif. This work demonstrates the suitability of the CRISPRi/dcas9 system in investigating genes putatively involved in anti-microbial resistance.

P15

Optimising extraction of RNA from intra-macrophage *Salmonella.*

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Abstract

Salmonella enterica are Gram-negative bacteria that cause disease in a broad range of host organisms. One hallmark of *Salmonella* pathogenesis is the ability of the bacteria to adopt an intracellular lifestyle within mammalian host cells. *Salmonella* expresses and produces large numbers of gene transcripts to facilitate metabolic remodelling and encode virulence factors that mediate the infection process. We have previously shown that *Salmonella* genes that are regulated during infection play important roles in adaptation, survival and proliferation within mammalian cells. Studying the *Salmonella* gene expression profile during infection thus offers a unique insight into *Salmonella* pathogenesis and the micro-environment experienced by the intracellular bacteria. Reliable intracellular gene expression profiling depends on the availability of high-quality bacterial RNA, free of host-cell DNA and RNA contamination. However, isolating bacterial RNA from infected cells is a difficult task. Our differential lysis method to isolate *Salmonella* RNA from infected mammalian macrophages and epithelial cells is technically challenging and often yields insufficient amounts of clean *Salmonella* RNA for reliable analyses.

The aim of my project is to evaluate the effectiveness of different methods to separate intra-macrophage *Salmonella* from lysed macrophages, to improve the reproducibility of our intracellular *Salmonella* RNA extraction protocol. I will present my latest findings that promise to improve the separation and recovery of intra-macrophage *Salmonella* RNA.

P16

Identification of possible cellular binding partners of the poxvirus immunomodulator fpv012.

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Abstract

Poxviruses are large double stranded DNA viruses with a characteristic morphology, undergo cytoplasmic replication and have a significant armoury of genes that are used to defend against the host immune response. The prototypic vaccinia virus (VACV), has well categorised genes known to immunomodulate the host immune system and, amongst this, is notorious in effectively antagonising the interferon (IFN) response.

Fowlpox virus (FWPV) is a member of the avipox family and is responsible for extensive morbidity and mortality amongst flocks infected. For our studies we used the highly attenuated FP9 live strain of FWPV, a successful vaccine vector in both avians and mammals.

Fowlpox virus gene 012 (fpv012) has previously been shown to partially block the poly(I:C) stimulation of the chicken IFN2 (equivalent to mammalian IFNβ) promoter (Laidlaw *et al*. 2013). This study aims to examine and demonstrate the possible interactions between fpv012 and cellular binding partners in chicken fibroblast cells using immunoblotting, immunofluorescence, immunoprecipitation and pull down approaches.

We identified the chicken serine threonine kinase (cTBK1) as a candidate binding partner via a SILAC-based tagged fpv012 pull-down method. In mammalian cells TBK1 is a key immune mediator in the classical IFN pathway. During viral infections it is responsible, with the IkB kinase-epsilon (IKKϵ), for the activation of interferon regulatory factor 3 (IRF3) and subsequent induction of IFNβ. VACV C6 protein has been shown to associate with TBK adaptor proteins (Unterholzner *et al*. 2011) and so this study will focus on the potential interaction between fpv012 and cTBK1.

P17

A comparison of the antibacterial effects of five essential oils and TCP antiseptic against the bacterial species *Staphylococcus aureus* and *Staphylococcus epidermidis.*

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Abstract

The aim of this study was to compare and evaluate the antibacterial effects of five essential oils (EOs) and the commercially available antiseptic TCP against the bacterial species *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* NCIMB 12721. Antimicrobial susceptibility screening was performed using the agar disk diffusion method. Both bacterial species showed susceptibility to tea tree, lavender, cinnamon and lemongrass EOs, with no observed susceptibility to grapeseed EO. Lemongrass EO showed the largest zones of inhibition (ZOIs) for both species (>40mm), followed by cinnamon and tea tree EOs (>25mm) taking into account the 6mm diameter of the filter paper discs. However, both species showed very little susceptibility when tested against the TCP antiseptic with small ZOIs (<8mm). Lemongrass and cinnamon EOs were selected for further testing to determine their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). The microtiter plate method was adopted to obtain the MICs for both EOs, and a spot inoculation was performed to determine the MBCs. A time-kill assay was implemented to determine the time points at which the EOs exhibited a bactericidal effect. In all instances, lemongrass oil showed a faster kill rate in comparison to cinnamon oil. Additionally, both bacterial species untreated and treated with lemongrass and cinnamon EOs were prepared for scanning electron microscope (SEM) imaging, to give a visual representation of the differences the bacterial cells undergo when treated with the EOs. In conclusion, lemongrass EO demonstrated the greatest antibacterial effect when tested against the selected bacterial strains.

P18

Investigating the efficacy of Lynronne-1D, a modified rumen microbiome derived antimicrobial peptide, with improved stability against Staphylococcus aureus.

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Abstract

Antimicrobial resistant infections are at a crisis point, posing a massive threat to human health with an anticipated mortality of 10million people by 2050. Antimicrobial peptides (AMPs) are a promising solution to treat drug resistant infections, and the rumen microbiome, a highly competitive ecosystem is a great resource for mining AMPs.

In this study, we aimed to improve the stability of a known rumen AMP with great therapeutic potential, Lynronne-1. This AMP is efficacious against topical skin infections of Methicillin resistant *Staphylococcus aureus* but has no activity in systemic infections when administered intravenously due to degradation by peptidases. To overcome this hurdle and improve its use intravenously, we substituted the L isoforms N and C terminal amino acid residues to D-isoforms, thereby increasing the stability of the peptide in the presence of trypsin by three-fold.

The activity of the modified peptide, named Lynronne-1D against *S. aureus* was subsequently investigated. Lynronne-1D retained its antimicrobial activity with an MIC of 8 μg/ml against *S. aureus* and improved MICs (>4-fold) in Gram-negative bacteria strains. The peptide had rapid and potent bactericidal activity causing ≥ 6log CFU/ml decrease in viable *S. aureus* cells within 30 minutes of treatment. It induced membrane permeabilization within 5 minutes and prevented biofilm formation by *S. aureus* cells. Lynronne-1D was also non-cytotoxic to mammalian blood cells. The improved properties of Lynronne-1D over the original peptide makes it a promising therapeutic agent for the treatment of systemic infections of *S. aureus*.

P19

Chemical warfare: How do rumen antimicrobial peptides regulate the rumen microbiome.

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Abstract

Antimicrobial peptides (AMPs) are part of microorganisms' chemical arsenal used to wage warfare against their competitors. However, AMPs may also act as probiotics regulating the biome to create a more biodiverse ecosystem.

Lynronne-1, is a recently isolated cationic, α-helical AMP discovered through functional metagenomics from plant attached rumen bacteria with antimicrobial activity against a variety of clinically important multi-drug resistant pathogens. Its efficacy especially against methicillin resistant *Staphylococcus aureus* infected wounds makes it a promising alternative therapeutic agent. However, the role of Lynronne-1 in the rumen environment is not fully understood. This study examines the antimicrobial and probiotic effect of Lynronne-1 within the rumen microbiome and its effects on the abundance of other plant colonising rumen bacteria.

The effect of MIC, sub-MIC and supra-MIC concentrations of Lynronne-1 on total bacteria, *Prevotella* spp, *Ruminococcus albus, Fibrobacter succinogenes* and *Ruminococcus flavefaciens* in unaltered rumen fluid was analysed by qPCR analysis. No significant changes were observed over a 24-hour incubation period. The lack of change could be due to the intrinsic resistance of rumen microbes against the AMP or its instability in rich rumen fluid. Lynronne-1 may also have a greater effect on non-bacterial microorganisms in the rumen microbiome and pathogenic organisms. Therefore, investigation into these groups of microbes may provide a better understanding of its role in the rumen. A more robust method such as RNAseq metatranscriptomics in addition to qPCR may be necessary for the accurate quantification of Lynronne-1's effect in the rumen.

P20

Investigating the biological substrates of ApeE, the*Salmonella* GDSL lipase autotransporter.

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Abstract

ApeE is an autotransporter protein that is conserved amongst all currently sequenced *Salmonella*. Previous work showed that ApeE is a GDSL lipase autotransporter protein that is able to cleave napthyl esters and expression of ApeE is induced upon phosphate limiting conditions. Autotransporter proteins, also known as type 5 secretion, have 3 functional domains; an N-terminal sec dependent signal sequence, secreted effector (passenger) domain and a β-barrel translocation domain. Evidence suggests that many autotransporters are important outer membrane proteins during Gram-negative pathogenesis. A recent study linked the phospholipase activity of an ApeE homolog to a potential role in virulence. As ApeE is upregulated in phosphate limited conditions, we wanted to investigate whether ApeE has phospholipase activity because to date, a biological substrate for ApeE has not been identified. Here, we use an *in vitro* recombinant protein system to determine biologically relevant substrates of ApeE and the Michaelis–Menten kinetics for these substrates, under different pH and determine the optimal conditions for enzyme activity. We show that ApeE is required for the growth of *Salmonella enterica* serovar Typhimurium in minimal growth medium with phospholipid as the sole carbon source and that ApeE can bind to host relevant lipids. These data indicate that ApeE could be important for the interaction of *Salmonella* with lipids derived from the host.

P21

Cathelicidin antimicrobial peptide paradoxically increases the growth and virulence of the pulmonary pathogen*Aspergillus fumigatus.*

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Abstract

The pulmonary mucus of cystic fibrosis (CF) patients displays elevated levels of the cathelicidin antimicrobial peptide LL-37 and the aim of this work was to assess the effect of LL-37 on the growth of *Aspergillus fumigatus*, a common pathogen of CF patients. Exposure of *A. fumigatus* to LL-37 and its derived fragment RK-31 (1.95 µg/ml) for 24 hours had a positive effect on growth (199.94 ± 6.172%, $p < 0.05$) and (218.20 ± 4.63%, $p < 0.05$) respectively, whereas scrambled LL-37 peptide did not. Exposure of 24 hour pre-formed mycelium to 5 µg/ml intact LL-37 for 48 hours increased hyphal wet weight (4.37 ± 0.23 g, $p < 0.001$) compared to the control and scrambled LL-37 treatments. Gliotoxin secretion was increased at 24 hours from LL-37 exposed hyphae (169.1 ± 6.36 ng/mg hyphae, $p < 0.05$) compared to the control and scrambled LL-37 treatments. Proteomic analysis of 24 hour LL-37 treated hyphae revealed an increase in the abundance of proteins associated with growth (eIF-5A, tissue degradation (aspartic endopeptidase) and allergic reactions (Asp F13). By 48 hour there was an increase in proteins indicative of cellular stress, growth and virulence. LL-37 and RK-31 pre-treated *A. fumigatus* display augmented virulence in an invertebrate model of invasive aspergillosis. These results indicate that LL-37 stimulates *A. fumigatus* growth and this may result in increased fungal growth and secretion of toxins in the lungs of CF patients.

P22

The Multiple Antibiotic Repressor (MarR) protein has one binding target in the Escherichia coli genome.

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Abstract

In order to respond to environmental stresses bacteria have evolved complex systems of global genetic regulation. One such system involves the multiple antibiotic resistance (mar) operon, which is negatively regulated by MarR. Environmental stresses, such as antibiotics, cause MarR to dissociate from the marRAB promoter. Currently, the genome-wide DNA binding properties of MarR are unknown. Here, we generated a set of genetic tools to allow 3x FLAG or 8x Myc tagging of a target protein at the N- or C-terminus. These tools were used to tag MarR and chromatin immunoprecipitation was used to show that MarR has a single binding target in the Escherichia coli genome.

P23

Exploitation of random transposon mutagenesis to reveal conditionally essential genes important for antibiotic resistance.

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Abstract

Insertion-site sequencing of a random transposon library is a high-throughput method used to link phenotype with genotype. It is commonly used to identify genes required for survival or pathogenicity of clinically relevant strains; or to understand poorly characterized organisms.

A mini-Tn*5* transposon library was constructed in *Escherichia coli* K-12 strain BW25113. The transposon library was sequenced by following an amended TraDIS protocol. A total of 904,084 unique transposon insertion points were identified. With an insertion every ~5 bp, this library is one of the most dense libraries published, and a valuable tool for whole genome screening.

The library was subsequently grown in LB supplemented with a range of antibiotics at sub-MIC concentrations. The conditionally-essential genes for each antibiotic were identified. Among this data, our findings have revealed phenotypes for a number of previously uncharacterised genes within the highly studied *E. coli* K-12 genome. Further work is underway to characterise the function of these genes.

Assigning function to previously uncharacterised genes improves both the annotation of *E. coli* K-12, but also aids the annotation of less well characterised organisms that have homologs of these genes. Finally, understanding the function of these genes may provide novel therapeutic targets that can be exploited in the design of new antibiotics.

P24

Characterising intragenic promoters and H-NS repression in *E. coli.*

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Abstract

The histone-like nucleoid structuring (H-NS) protein is responsible for repressing transcription that initiates spuriously within the coding sequences of AT-rich genes. In this work, we sought to better understand why promoters occur so frequently within AT-rich DNA. To do this, we compared the properties of i) canonical promoters ii) promoters within H-NS bound genes and iii) promoters generated by random combinations of nucleotides. We show that randomly generated promoters, and promoters within AT-rich genes, differ from canonical promoters in several ways. In particular, spurious promoters are often dependent on AT-tracts upstream of the promoter -10 element. These AT-tracts play a key role by altering DNA curvature and facilitating non-specific interactions between the promoter and RNA polymerase sigma factor.

P25

3D Polymer scaffolds for industrial biocatalysis.

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Abstract

The project is developing robust biocatalysts in the form of enzymes expressed in biofilms. The aim is to design functionalised polymer scaffolds onto which biofilm forming bacteria adhere in a controlled manner, to form protected communities that can be used in reactors. Poly(acryloyl-hydrazide) has been chosen as the basic polymer scaffold, and its easy post-polymerisation modification results in highly functional polymers. To date, the polymer has been functionalised with a range of nitrogen heterocyclic aldehydes predicted to interact with bacteria. Clustering of the biofilm-forming *Escherichia coli* strain PHL644 has been analysed upon mixing with differently functionalised polymers. When bacteria is suspended in buffer, the polymers induce clustering of cells. However, when mixing is performed in water or NaCl solution, clustering is significantly decreased. It's likely that in the absence of buffer, the relatively basic aldehydes become protonated whereas in the presence of buffer, remain in their native state. Hydrophobicity of a surface encourages bacterial adhesion so it's possible that the more hydrophobic, neutral polymers are inducing PHL644 clustering. Adhesion is the first stage of biofilm formation. Further experiments have been performed to investigate induced biofilms; crystal violet binds to biofilm components and preliminary results of these assays indicate greater induction of biofilm by hydrophobic polymer. The future direction of this project is yet to be decided, but depending on the success of these hydrophobic polymers to induce controlled biofilms, it could be possible to design 3D polymer scaffolds to further optimise control over biofilm formation.