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S1/P1

Novel tripartite tricarboxylate transporters from Rhodopseudomonas palustris

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Rhodopseudomonas palustris is a soil non-sulfur purple bacterium, with ability to degrade lignin-derived compounds and also to generate high yields of hydrogen gas, what raises several biotechnological interests in this bacterium. Degradation pathways, though, must begin with substrate uptake. In this context, Soluble Binding Proteins (SBP`s) dependant transporters are responsible for high-affinity and specificity substrate uptake. The aim of this study is to characterize the Tripartite Tricarboxylate Transporter (TTT) family network in this organism, which might serve as a new source of diversity for biotechnologically interesting uptake systems. Three different SBP dependent secondary transporters of the TTT family have been characterized so far, named R1, R2 and R3. The strategies to characterize them involve a range of different techniques: Bioinformatics, Protein crystallization, Differential Scanning fluorescence, Tryptophan fluorescence and mass spectrometry. R2 was found to bind to C4 dicarboxylic acids, having lower affinity for succinate and fumarate and higher affinity for malate. Q-PCR assays revealed 2.5 to 3 times overexpression of R2 under growth in these carbon sources. For R1 and R3, although genetic analysis suggested they might be involved in glutaconic and protocatechuic acid uptake, respectively, binding assays performed did not support this hypothesis. R1 was successfully crystalized and we have solved its structure at less than 2 angstroms resolution. The structure shows a ligand-free open conformation, in a classical Venus flytrap formation. Comparison with other secondary transporter`s SBP shows R1 lacks residues which are key for known substrate coordination and may thus bind to novel types of substrates.

S1/P2

The D408A substitution in the AcrB multi-drug efflux protein of *Salmonella* Typhimurium SL1344 confers loss of function

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Salmonella Typhimurium is one of the most common causes of foodborne disease. As with many other pathogens, antibiotic resistance in *S.* Typhimurium has complicated its treatment, leading to increased morbidity and mortality worldwide. Many Gram-negative bacteria are inherently resistant to some antimicrobials due to multi-drug efflux pumps; AcrAB-TolC complex is the main efflux pump in Enterobacteriacea. AcrB is the pump in this complex and it is conserved between bacterial species, with an average of 78.5% identity between the DNA sequences and approximately 80% similarity between the amino acid sequences amongst Enterobacteriaceae. This pump acts as a drug-proton antiporter, four residues have been previously reported as essential for proton translocation in *Escherichia coli* AcrB: D407, D408, K940 and T978. AcrB of *E. coli* has an identity of 86% and a 94% similarity to that of *S.* Typhimurium. Based on these data, we constructed an AcrB D408A chromosomal mutant in *S.* Typhimurium SL1344. Western blotting confirmed that the mutant had the same level of expression of AcrB as the parental wild type strain. The mutant had no growth deficiencies either in LB or MOPS minimal media. However, compared with wild type SL1344, the mutant had decreased efflux activity and was multi-drug hyper-susceptible. Interestingly, the phenotype of the AcrB D408A mutant was almost identical to that of an Δ*acrB* mutant. These data indicate that a single point mutation affecting proton translocation of AcrB renders the pump inactive in *Salmonella*.

S1/P3

Purification and characterisation of multidrug resistance PACE family efflux proteins

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Active multi-drug efflux pumps are one of the major mechanisms of bacterial resistance to drugs and are an alarming threat to antibiotic therapy. From the widespread presence of these pumps in bacterial pathogens, five families of multidrug efflux systems have been identified. These are the MFS (major facilitator superfamily), SMR (small multidrug resistance), ABC (ATP-binding cassette), MATE (multidrug and toxic efflux), and RND (resistance nodulation division) families [1]. The novel membrane protein AceI (*Acinetobacter* chlorhexidine efflux) from the Gram negative bacterium *Acinetobacter baumanii* conferred resistance to chlorhexidine [2, 3]. Recently 23-homologues of the AceI protein were grouped in a new sixth family of bacterial multidrug efflux pumps, designated the Proteobacterial Antimicrobial Compound Efflux (PACE) family [4]. We are undertaking the expression screening, purification and characterization of PACE family proteins. Starting with eighteen proteins, small-scale expression screening identified six (STY_3166, Fbal_3166, PFL_4558, A1S_1503, Tmarg_opt and PSPTO_3587) that were suitable for scaling up to larger cultures, inner membrane preparations and purifications. AceI and four of the homologues were purified and their integrity analysed using CD spectroscopy, which revealed high contents of alpha helix. Fluorimetric measurements showed that the widely used biocide chlorhexidine bound to AceI, STY_3166 and Fbal_3166.

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S1/P4

Improving transport capacity of Miscanthus-derived sugars and sugars acids by Escherichia coli

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There is increasing demand for biorenewable energy that can be produced using plant biomass as the source of carbon and energy for the bacteria production of valuable chemicals and biofuels. One potential biofuel crop of the grass *Miscanthus* which can be processed to release lignocellulosic fractions for bacterial fermentation. The carbon and energy sources in this feedstock contain both hexose and pentose sugars as well as sugar acids. The efficient utilisation of all of these compounds by the fermenting bacterium is important to ensure maximal conversion of biomass to product. Here we have studied the ability of bacteria to transport the *Miscanthus* hemicelluloses components xylopyranose, arabinofuranose and glucuronic acid, using an open-source ethanol-producing strain of *Escherichia coli*. We report data on the construction of *E. coli* strains to identify novel transporters for these sugars and our work in the identification of a novel family of furanose-specific ABC transporters called Gaf transporters that are likely important for efficient uptake of arabinose, which is found exclusively in the plant hemicelluloses as the arabinofuranose form.

S1/P5

Development of flavonoid therapeutics: transport mechanisms in the model eukaryote *Dictyostelium discoideum*.

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We are investigating the function of two members of the multidrug and toxin efflux (MATE) transporter family, which are ubiquitous throughout all living kingdoms. MATEs have a wide range of substrate targets, with transporters conferring the ability to facilitate the movement of single or many compounds (Omote et al., 2006).

We have identified two genes encoding 'MATE' proteins in the model amoeba *Dictyostelium discoideum*, and are making knockout and reporter lines for physiological and imaging studies. Expression of the transporters at different life stages suggests the two have distinct and not redundant functions. The first transporter gene is transcribed in highest levels when cells signal to each other and aggregate. The second transporter gene is predominant earlier, when unicellular Dictyostelium cells prey on bacteria, and transcription also peaks later when cells have aggregated to form a motile, multicellular slug.

We hope to address fundamental questions in the cell biology of this important model organism such as the role of the extracellular matrix and how it is formed; to study localisation of these proteins in the unicellular and multicellular life cycle; and also to contribute to work on the mechanisms of a flavonoid therapy for polycystic kidney disease which is under development by our collaborators (Waheed et al., 2014). In plants, it is hypothesised that so-called 'MATE' transporter proteins may be involved in flavonoid transport: currently there is sparse literature on the transport mechanisms that would allow/prevent this family of compounds reaching eukaryotic cellular targets.

S1/P6

Pulling on a plug domain: direct mechanical gating of BtuB, an outer-membrane protein transporter.

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TonB-dependent transporters (TBDT) are outer-membrane proteins of Gram-negative bacteria responsible for the scavenging and import of scarce metallo-organic complexes from the environment. The transport process, which occurs against a concentration gradient, requires the presence of the TonB-ExbB-ExbD complex within an energised inner membrane. TonB, tethered to the inner membrane, can span the periplasm and interacts with the N-terminal Ton box motif of a TBDT that is released from the plug domain that occludes the lumen of the TBDT upon binding of substrate. Although it is well accepted that formation of this complex results in opening of the lumen, the mechanism by which this occurs is still unknown. The widely accepted 'pulling model' posits that the plug domain is mechanically remodelled upon complex formation. However, despite its acceptance, no direct experimental evidence supports this theory. Here, atomic force microscopy is used to reveal that the TonB:Ton box interaction is strong enough to partially unfold the plug domain of the vitamin B12 transporter BtuB, creating a channel through the receptor before complex dissociation. We also show that the amount of unfolding is independent on the amount of force applied to the interaction - a feature which prevents the entire receptor from unfolding when large forces

are used. Using a combination of disulphide cross-linking experiments to locate the unfolding region along with a vitamin B12 uptake assay and MD simulations, the presented work demonstrates that, in addition to lipid-induced gating, membrane transport channels can also be activated by protein remodelling-induced mechanical gating.

S1/P7

Using bacteria to fight bacteria: Paratization of ferredoxin-uptake receptors in Pectobacterium

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Antibiotic resistance is a global problem that affects both health and food security affecting 800 million people every year. Gram-negative plant pathogens, such as Pectobacterium, have a detrimental effect on crop yields. Bacteriocins are protein antibiotics that are usually active only against bacteria closely related to the producing strain; these bacteriocins are often species specific and therefore offer an alternative to the broad spectrum antibiotics used in clinical practice. Three recently described bacteriocins produced by P. carotovorum consist of a [2Fe-2S] plant-type ferredoxin domain fused to a bacteriocin cytotoxic domain and these parasitize an existing ferredoxin uptake system to gain entry into target cells. The normal physiological role of this uptake system is to acquire iron from plant ferredoxin. We have identified the ferredoxin/pectocin receptor as a TonB-dependent receptor that we have designated FusA. Bioinformatic analysis indicates the existence of a ferredoxin uptake operon, which in addition to the receptor FusA, encodes a protease, an ABC-transporter which likely act to cleave ferredoxin and transport liberated iron to the cytoplasm and a homologue of TonB. We are currently dissecting the roles of these proteins in ferredoxin uptake using a combination of structural (SANS and SAXS) and functional studies.

S1/P9

S-layer Biogenesis of Clostridium difficile and the Accessory Secretion Pathway

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Clostridium difficile infections are a major problem within health care settings, commonly occurring after damage to the intestinal microbiota through the use of antibiotics. The toxins of *C. difficile*, responsible for most of the disease pathology, are well characterised. However, other virulence determinants of *C. difficile* are poorly understood.

The S-layer is a proteinaceous paracrystalline array that completely coats the bacterial cell and has been shown to be essential for virulence in *C. difficile*. The S-layer is mostly composed of two proteins created through the cleavage of the pre-protein SlpA. *slpA* has been shown to be essential for growth. However, little is known about the function and biogenesis of SlpA and the S-layer.

In bacteria, the majority of secreted proteins pass through the Sec secretion pathway. This pathway is characterised by the protein channel SecYEG and the ATPase SecA. SecA mediates transduction of the secreted peptide through the channel through conformational changes linked with ATP hydrolysis. Many Gram positive bacteria such as *C. difficile* have recently been shown to contain an additional Sec pathway characterized by the presence of an additional SecA protein. In these systems the two Sec proteins have non-redundant functions, with SecA1 showing housekeeping functions and SecA2 secreting a small sub-set of proteins. SecA2 of *C. difficile* has been shown to be responsible for secretion of SlpA and is thought to secrete the other proteins of the S-layer.

This project aims to probe the localization of SecA2 secretion and how this contributes to S-layer biogenesis.

S1/P10

Phylogenetic analysis of bacterial Ptr peptide transporters reveals significant gene family expansion in the Enterobacteriaciae

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The PTR family of peptide transporters, also referred to as the proton dependent oligopeptide transporter or POT family, are proton-coupled peptide transporters and members of the Major Facilitator Superfamily (MFS) of secondary active transporters. The members of this family are found in all domains of life and have important roles in the nutritional acquisition of peptides. The model bacterium Escherichia coli K-12 has 4 PTR transporters, which are encoded by the genes dtpA-D, but with a few exceptions there has been little wider analysis of the distribution and evolution of the PTR transporters in bacteria. During a Harry Smith vacation studentship, a collection of over 200 bacterial PTR sequences were collected, curated and used to generate a maximum likelihood (ML) tree of the MUSCLE-aligned proteins. Through analysis of the structure of the tree combined with gene synteny analysis of the dtp genes, we were able to define 3 additional subfamilies of PTR transporters in bacteria that we have named DtpE-G and establish more accurately the relationships between the know DtpA-D families. We also report analysis of the distribution of two 'accessory' helices, HA and HB, that are often found in bacteria but which have clearly been lost in a distinct number of clades over evolutionary time. These data help better define the evolution and potential functional diversity of this important family of peptide transporters in bacteria.

S1/P13

Exploring the structure and function of the ligand binding domain of the antimicrobial peptide transporter BceAB of *Bacillus subtilis*

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Resistance of low GC-content Gram-positive bacteria to antimicrobial peptides is often mediated by unique resistance modules consisting of a two-component regulatory system and an ATP binding cassette (ABC) transporter. Intriguingly, the transporter not only provides resistance but also plays an important role in sensing of antimicrobial peptides. These BceAB-like transporters possess a characteristic large extracellular domain of about 200 amino acids, which was found to be essential for peptide sensing and is thought to determine substrate specificity. We therefore hypothesise that this domain harbours the ligand binding site of the transporter. Interestingly, there is no significant sequence conservation in this domain, even between transporters that recognise the same substrates, and we therefore propose that ligand specificity is determined on the structural level. We here applied biochemical and biophysical approaches to study ligand binding by the extracellular domain of the transporter BceAB of *Bacillus subtilis*. Our results show that this domain is indeed capable of direct substrate binding, which is accompanied by conformational changes in the protein. These findings not only provide direct evidence of ligand binding but also pave the way for structural investigations to elucidate the mechanism of substrate recognition by these unique resistance systems.

S1/P14

Impact of OmpR on the uptake of oligogalacturonides in Yersinia enterocolitica: activation of the outer membrane porin KdgM1

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Bioinformatic analysis of the enteropathogen *Yersinia enterocolitica* genome revealed genes involved in pectin degradation and catabolism that are organized similarly to those in the genome of *Dickeya dadantii*, a phytopathogenic bacterium. Proteins KdgM1 and KdgM2 of *Y. enterocolitica* are homologs of *D. dadantii* specific oligogalacturonate porins KdgM and KdgN, that overlap functionally and their expression is controlled by KdgR repressor. We previously showed that the transcriptional regulator OmpR negatively influences *kdgM2* expression in *Y. enterocolitica* O:9. The aim of this study was to investigate the molecular mechanisms regulating *kdgM1* expression.

To examine whether the expression of *kdgM1* is under the control of OmpR, the chromosomal transcriptional fusion *kdgM1-lacZYA'* was constructed in the wild-type strain, Δ*ompR*, Δ*kdgR* and Δ*ompR*Δ*kdgR* mutant derivatives. The gel shift assays (EMSA) was used to detect OmpR complexes with the regulator sequences of *kdgM1*.

Based on measurements of β-galactosidase activity we found that *kdgM1* is under KdgR repression. Moreover, the lower *kdgM1* expression was observed in the Δ*ompR* mutant compared to the wild-type strain, in the *kdgR* mutant background. Inspection of the regulatory region of *kdgM1* showed one putative OmpR binding site with 50% identity to the *E. coli* consensus sequence. EMSA showed that OmpR binds to the promoter region of *kdgM1*, indicating that this factor directly regulates the transcription of this gene.

Our results revealed the involvement of OmpR in the direct, positive regulation of KdgM1 and indicated that *kdgM1* and *kdgM2* expression in *Y. enterocolitica* is subject to the reciprocal OmpR regulation.

S1/P15

Investigating the role of the *spoVA* operon in *Clostridium difficile* endospores

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Bacterial spores are among the most resilient forms of life, capable of surviving exposure to UV radiation, extreme temperatures and disinfectants. Such properties enable endospores of the pathogenic bacterium *Clostridium difficile* to persist in healthcare facilities and act as the infectious agents of *C. difficile* associated disease (CDAD). Upon ingestion by susceptible individuals, these spores arrive in the colon where they germinate to form toxin-producing vegetative cells. The resulting CDAD is responsible for over 14,000 deaths annually in the US and is a substantial economic burden on healthcare facilities worldwide.

Associated with spore resilience is the presence of dipicolinic acid (DPA) in the spore core. Previous studies in other *Firmicutes* have shown that DPA entry into the forming spore during sporulation, and subsequent release during germination, is mediated by the *SpoVA* proteins. The spoVA operon varies in the number of constituent genes between spore-forming *Firmicutes*, with seven in the model organism *Bacillus subtilis* and only three (*spoVAC*, *spoVAD* & *spoVAE*) present in *C. difficile*. The products of this operon are thought to bind DPA and form a channel in the spore inner membrane through which DPA translocates.

Here we have used allelic exchange to create a *C. difficile spoVA* in-frame deletion mutant and also to complement the mutation in the chromosome. Spores of the mutant and complement have been characterised to investigate role and function of the *spoVA* operon in *C. difficile*.

S1/P16

Structure-function characterisation of *Chlamydia pneumoniae* Major Outer Membrane Protein (MOMP)

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Major outer membrane proteins (MOMPs) are structurally and immunologically dominant proteins in the Chlamydia outer membrane but their detailed characterisation remains a major challenge, due to difficulties in producing recombinant protein in a folded, active state, which has significantly hindered recombinant vaccine approaches. To date, MOMP's structure has been solved to a resolution of 4Å using non-optimised crystals. Currently there is very little literature surrounding structure-function characterisation of Chlamydia MOMPs. Functional assays are in progress to assess MOMP's potential activity as a fatty acid transport, due to its close resemblance to fatty acid transporter FadL of *E.coli*. We have collected SRCD and FTIR data on MOMP to offer more detailed insight into its structure-function properties, in addition to electrophysiology to assess its role as a porin. Chlamydia's apparent lack of peptidoglycan suggests an alternative mechanism is utilised in order to confer stability. Due to the large number of cysteine residues within MOMP, it has been suggested that formation of disulphide bonds between MOMP and other cysteine rich proteins may provide such stability. Future work will also be focussed on using computational methods to hypothesise MOMP's organisation within the outer membrane.

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S1/P17

The Chaperone:Usher Translocon of Yersinia pestis F1 Capsular Antigen

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E. coli and other Gram negative bacteria coat themselves in remarkably stable protein fibres assembled via the well-recognised chaperone-usher (CU) pathway. The simplest of these are long polymers, like the F1 fibres of Yersinia pestis, and these represent an appealing scaffold structure to display short foreign peptides. The potential of F1 fibres to act as a carrier of short peptides was tested by replacing loops within the Caf1 subunit with Gly residues, charged residues or polyhistidine. Permissive and non-permissive sites were identified. The Caf chaperone-usher translocon was modelled based on the high resolution structures of the related E. coli FimD usher, periplasmic domains of the Caf1A usher and the Caf chaperone-subunit complex. The translocon model was used to understand properties of the usher barrel and identify problems in export of modified fibres. This was used to aid design of a modified usher which was subsequently shown to increase export of a mutate F1. Thus this study demonstrated flexibility of the Caf CU pathway for surface display of different peptides and the ability to use the translocon model to identify problem areas and optimize export.

S3/P1

Title: Studies on the functional significance of asparaginase and glutaminase of *Klebsiella pneumoniae*. Author: Rashed Alghamdi, (ra321@le.ac.uk)First year PhD student (infection and Immunity) at University of Leicester,

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Klebsiella pneumoniae is a causative agent of several nosocomial and community acquired infections. However, what controls *K. pneumoniae* virulence is incompletely understood. It has been suggested that efficient acquisition and metabolism of host nutrients in the sites of infection are important for the microbe's ability to cause infections. For example, asparaginase and glutaminase are sets of enzymes that assist *K. pneumoniae* in acquiring necessary nitrogen sources when ammonia (NH3), the preferable nitrogen source, is low < 1 mM or absent. Asparaginase and glutaminase are involved in the hydrolysis of L-asparagine and L-glutamine to L-aspartate and L-glutamate, respectively, which releases NH_3, the preferred nitrogen source for the microbe. *K. pneumoniae* is also able to use L-asparagine and L-glutamine as the sole nitrogen and carbon sources *in vitro*. It has been found that *K. pneumoniae* contains four putative asparaginase and glutaminase genes (*yneH, ybiK, ansA* and *KPN_01165*). The aim of this study was to investigate the contribution of these enzymes in *K. pneumoniae* KR3167 survival and virulence by creating isogenic mutant strains in these genes. Growth studies showed that wild type (WT) *K. pneumoniae* has efficient mechanisms to grow in M9 medium supplemented with glucose and either L-asparagine or L-glutamine. In terms of the mutants, there was no significant difference in growth between WT and Δ*yneH*. However, there were significant difference between WT and the *ansA, ybiK,* and *KPN_01165* mutants. Enzyme activity assays showed that the strains Δ*KPN_01165*, and Δ*ybiK* had lower asparaginase activities under the growth conditions compared to the WT.

S3/P2

Assembly of the Cytochrome c Oxidase in Campylobacter jejuni

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Campylobacter jejuni, a foodborne microaerophilic pathogen, is a serious matter of concern worldwide. It uses complex cytochrome-rich respiratory chains for growth and host colonisation. The respiratory chains are composed of many enzymes found in the inner membrane and periplasm, and includes a pathway to a cbb3-type cytochrome c oxidase that transfers electrons to dioxygen. In C. jejuni, genes cj1487c, cj1488c, cj1489c and cj1490c collectively encode the CcoNOQP subunits of the oxidase. This type of oxidase contains two c-type cytochrome subunits and a bi-nuclear haem-copper active site consisting of Cu(B) and b/b3 type haems. Insertion of Cu(B) normally requires an assembly system of copper chaperones. In C. jejuni genes cj0911, cj0910, cj0909 and cj0908 may encode proteins needed to transfer Cu from the periplasm to the CcoN subunit; Cj0911 is homologous to bacterial Sco proteins known to have a Cu chaperone function, but the role of the other proteins is unknown. Objective: We are investigating the roles of cj0911, cj0910, cj0909 and cj0908 in cbb3 oxidase assembly. Methods and Results: We have made a series of individual and multiple gene knock out mutations in C. jejuni, which have been phenotypically characterised using growth assays in limited copper media. Assessing oxygen consumption by each mutant using oxygen electrode experiments should provide further insights into copper utilisation. Conclusions: These findings will help us to understand the functioning of copper binding proteins and ultimately will allow us to understand complex cytochrome-rich respiratory chains within C. jejuni and their role in growth and host colonisation.

S3/P3

Green tea and green tea based nanoparticles as a potent antibiotic

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Recently researchers have demonstrated that green tea's catechins such as epicatechin -3-galat (ECG), epigallocatechin (EGC) and epigalocate chin-3- gallat (EGCG) have a broad activity as antibiotic (Gram positive, Gram negative) antiviral and antifungal. In addition some studies have shown that green tea based nanoparticles with carrying other antibiotics not only increase efficacy of different groups of antibiotics but also decrease drug resistance in MRSA against B-lactams. In this review we have shown the potential of green tea and it's nanoparticles in prevention and treatment of infectious disease.

Key words: green tea, nanoparticle, antibiotic

S3/P4

MbfA, a Novel Iron Export System Involved in *Brucella* Pathogenicity and Intracellular Survival?

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The *mbfA* (**m**embrane **b**ound **f**erritin **A**) gene is carried by all *Brucella* species and is widely conserved in α-proteobacteria. *mbfA* encodes a novel, chimeric protein (MbfA) comprising an N-terminal peroxide reductase (erythrin) domain fused to a C-terminal, membrane-embedded, iron-export protein (VIT1, **v**acuolar **i**ron **t**ransport **1**). Both domains are anticipated to contribute to oxidative-stress resistance and thus MbfA could play an important role in the intracellular survival of *Brucella*. To further understand the function of MbfA, the soluble erythrin domain was isolated and a di-iron center (likely coordinated by a conserved iron-binding motif) was successfully incorporated in vitro; the resulting di-Fe erythrin exhibited strong hydrogen peroxide decomposition activity *in vitro* supporting a role in redox-stress defense.

A single nucleotide substitution in the proximal coding region of the *mbfA* gene renders this gene cryptic in *B. melitensis*, as confirmed by Western blotting. Thus, a *B. melitensis mbfA* mutant exhibited no additional phenotype whereas a *B. suis mbfA* mutant displayed enhanced sensitivity to both Fe^{2+}- and H_2O_2-mediated oxidative stress. Macrophage survival assays showed that absence of functional *mbfA* decreases intracellular survival during the early intracellular colonization phase. This suggests reduced capacity to resist the acidification and redox stress of the endosomal and transient lysosomal compartments in the macrophage, which precede occupation of the **r**eplicative **B**rucella **c**ontaining **v**acuole (rBCV). The results are therefore consistent with a role for *mbfA* in intracellular survival and redox stress resistance, and suggest that *B. melitensis* experiences less exposure to redox stress than other *Brucella* spp. during cell invasion.

S3/P5

Epidemiology and treatment of MRSA in Jeddah hospitals

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Staphylococcus aureus is the most frequently isolated pathogen in hospitals and community and MRSA prevalence has increased in Saudi Arabian hospitals from 2% in 1989 to 33% in 1998. In this study, the prevalence of healthcare associated infections and the predominant organisms was investigated through survey of the infection control departments in three hospitals in Jeddah, namely KAUH, KFH, and MCH. 207 MRSA isolates were collected from the three respective laboratories and their MAR profiles identified. 23 strains were selected for further investigation using PFGE and Spa typing based on MAR profile and demographic data. In addition, the antimicrobial activity of some Saudi herbs and spices were assessed in vitro and in vivo. Data showed there was a similarity in infection control policies across the three hospitals. Collective data showed that MRSA was frequently isolated from wounds (36.7%) and RTI (30.4%). They were resistant to Penicillin (100%), Oxacillin (100%), E (62.9%), Gn (51.2%), trimethoprim-sulfamethoxazole (54.6%) and cipro. (50.2%) 100% sensitive to Vancomycin, teicoplanin and Rifampicin. Ten MAR profiles were identified. Two were endemic in KAUH and KFH and resistant to 6/9 antibiotics. One MAR profile was endemic in MCH and showed resistance to 5/9 antibiotics. The majority of strains were PFGE type 1 predicted as EMRSA1 and spa type t-363, t-037 and these were seen in the 8/9 MAR profiles. The MIC, MBC and time kill assays of aqueous and alcoholic extracts of some herbs were active at 512mg/ml. GC-MS analysis confirmed the major components in extracts were phenolic group.

S5/P1

The vSAα specific lipoprotein like cluster (*lpl*) of *S. aureus* USA300 contributes to immune stimulation and invasion in human cells

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Staphylococcus aureus is a member of *Staphylococcus* genus and causes a wide range of diseases including normal skin infection, food poisoning or other deadly illnes, of which, methicillin-resistant *S. aureus* (MRSA) is one of the major causes of hospital-acquired infection worldwide. The *S. aureus* USA300 strain, a clone of community-associated methicillin-resistant *S. aureus* (CA-MRSA), was firstly isolated in 2000 and had effectively epidemic infection in North America and Europe, that accounts for more than 50% of all disease caused by the entire *S. aureus* species. USA300 genome consists of vSAα and vSAβ islands located in chromosome and shared the common origin but exhibit differences in gene content that are proposed to possess functional roles in pathogenesis. USA300 contains 65 predicted lipoprotein genes, among them, 10 lipoprotein-like (*lpl*) genes located in vSAα islands.

To analyze the function of the *lpl* cluster in USA300, a marker-less deletion mutant was constructed. The mutant was affected in stimulation of pro-inflammatory cytokines in human monocytes and keratinocytes that was due to the encoded lipoproteins. However, most important was the finding that the *lpl* cluster contributed to invasion into human keratinocytes and mouse skin; which is probably also the reason why in a mouse kidney abscess model the bacterial burden of the kidneys was lower in the mutant compared to wild type. An increased internalization into host cells contributes to persistence and relapsing infections; therefore, the *lpl* cluster is probably the reason for the high spreading and epidemic activity of the clonal complex 8 lineages.

S5/P3

Mechanisms of differential virulence between *Streptococcus pneumoniae* and *Streptococcus mitis*

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Streptococcus pneumoniae and *Streptococcus mitis* are naso-oropharyngeal commensals that are genetically similar. However, *S. pneumoniae* is highly pathogenic and a common cause of pneumonia and septicaemia, whereas *S. mitis* rarely causes disease. We hypothesise that differences in sensitivity to innate immunity may underlie these differences in virulence phenotype. We compared sensitivity of *S. pneumoniae* and *S. mitis* to neutrophil killing. After opsonisation with serum but not with heat-treated serum or PBS, *S. mitis* was markedly more sensitive to neutrophil killing compared to *S. pneumoniae*. These differences suggested *S. mitis* was relatively complement sensitive, and flow cytometry assays of C3b/iC3b deposition confirmed there was increased complement opsonisation of *S. mitis* compared to *S. pneumoniae*. *S. pneumoniae* resistance to complement is partially dependent on binding of the immune regulator Factor H by the surface protein, PspC. We investigated Factor H binding to S. mitis using flow cytometry. The results demonstrated that there was no significant factor H binding to *S. mitis*. By inserting *pspC* of *S. pneumoniae* into *S. mitis*, we demonstrated that expression of PspC enabled *S. mitis* to then bind Factor H. Investigation of C3b/iC3b confirmed a decrease in opsonisation. Furthermore, survival in whole human blood of this modified strain showed an increase, when compared to the wild-type strain. These results suggest that an inability to bind factor H might underpin *S. mitis* sensitivity to opsonisation with complement and neutrophil killing compared to *S. pneumoniae*, and therefore contribute to the differences in virulence between these two commensal species.

S5/P4

Toxicity evaluation of different *Cronobacter sakazakii* isolates in human cell lines

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Cronobacter sakazakii is an opportunistic pathogen that has been implicated in a number of infections, in particular, neonatal meningitis and necrotizing enterocolitis (NEC). Knowledge of the mechanisms of *C. sakazakii* infections to date has focussed on bacterial invasion, colonisation and stimulation of the host inflammatory response. However, there is relatively little information on the susceptibility of intestinal and brain cells to bacterial cytotoxicity resulting in cell damage. This study was focused on potential pathogenic mechanisms by which *C. sakazakii* has the capacity to cause cytotoxicity to both human

intestinal (Caco-2) and human brain (HMGC, HBMEC) cells. Five fatal necrotizing and five meningitis isolates from fatal outbreaks have been chosen. These findings indicate that there is a strain to strain differences in cytotoxicity potential. Four strains 767, 701, 696, 694 showed a significant metabolic reduction in Caco2 cells and high level of lactate dehydrogenase release when compared to negative controls. These isolates recovered from different clinical sources during an outbreak belong to the same sequence type (ST4) and same pulsotype. It is interesting to find that strains 2106 and 2107 isolated from the blood samples of the same patient from a separate outbreak caused fatal NEC although revealed a different sequence MLST profile and showed a differ-ent result in attachment, invasion and cytotoxicity assays. Consequently, this study proved that *C. sakazakii* is capable of causing variable cytotoxicity to Caco2, HMGC and HBMEC cells however there are variations of this cytotoxicity level dependent on cell line and sequence type.

S5/P5

Investigating the potential pathogenicity of *C. malonaticus* using three human cell lines

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The *Cronobacter* genus, which belongs to the *Enterobacteriaceae*, consists of 7 species in total, of which 3 are clinically relevant; *C. sakazakii*, *C. malonaticus* and *C. turicensis*. Of these *C. malonaticus* has received less attention as it was associated with non-life threatening adult infections. However recent reports have shown that *C. malonaticus* may on occasions be linked to serious infections in neonates and thus it became more important than before (Asato et al., 2013; Hariri et al., 2013). *Cronobacter* first colonises the human intestine and subsequently invade and cross to the blood stream to initiate systemic infection. Three human cell lines Caco-2, U937 macrophage and human brain microvascular endothelial cells (HBMECs) have been used to test the pathogenicity of 20 *C. malonaticus* strains. The results of adhesion and invasion into Caco-2 showed that all strains can attach to this cell line. However just 3 strains were highly invasive into Caco-2, four were not able to invade and the rest showed moderate ability. Survival and replication assay into U937 macrophages showed that some strains were able to survive and replicate during 48 hours while others were either not taken up or killed. There was a lack of correlation between adhesion and invasion into the HBMEC cell line, as not all high attached strains could invade. Three strains were highly invasive while 2 strains were moderate and others were low or could not invade. The results of this work confirm the potential of *C. malonaticus* to cause serious infections in neonates.

S5/P6

ERK5 Signalling Regulates Macrophage Vomocytosis of Cryptococci

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The opportunistic fungal pathogen *Cryptococcus neoformans* is the known etiological agent of the life threatening disease cryptococcosis, responsible for over half a million human deaths per annum. Professional phagocytes, such as alveolar macrophages, phagocytose inhaled spores and attempt to destroy the pathogen, however this process is inefficient in the immunocompromised. In these patients, the macrophage behaves like a "Trojan Horse" acting as both a cryptococcal dissemination vector and a protective niche against antifungal agents/cells present in circulation.

Vomocytosis is a non-lytic expulsive mechanism whereby *C. neoformans* exits the macrophage leaving both pathogen and host macrophage with a morphologically normal phenotype. The clinical implications of vomocytosis are poorly understood but it is hypothesised to reduce the pro-inflammatory immune response that would be induced via macrophage lysis, hence enhancing pathogen survival. Regulating the rates of vomocytosis in vivo may have dramatic consequences on disease progression. For instance, enhancing the rate of vomocytosis in vivo might allow other antifungal cells and molecules to destroy the freshly released cryptococci, hence reducing pathogen burden and improving prognosis.

Using a combination of pharmacological inhibitors and genetic approaches, we now demonstrate a key role for the atypical MAP-kinase ERK5 in regulating cryptococcal vomocytosis. By modifying ERK5 activity pharmacologically, we were able to increase the rate of vomocytosis in both murine and primary human phagocytes without modifying cryptococcal growth, intracellular proliferation rate or macrophage cytokine signalling. Such an approach thus offers a potentially powerful route to subtly modify the host-pathogen interaction during systemic cryptococcal infection.

S5/P7

Characterisation of a putative *tfs4* ICE-encoded toxin-antitoxin system in *H. pylori*

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Type II bacterial toxin-antitoxin (TA) systems are composed of a stable toxin and a cognate labile antitoxin that inhibits toxin activity. TA systems have been proposed to play roles in bacterial persistence, stress regulation and antibiotic resistance. *Helicobacter pylor*i is a stomach-dwelling, spiral shaped, Gram-negative bacterium that infects ~50% of the world's population. Persistent infection is linked with chronic gastritis, peptic ulcer disease and gastric cancer. A putative bicistronic TA operon (HPP12_0452-HPP12_0453) was identified in the *tfS4*

virulence-associated integrating conjugative element (ICE) present in some *H. pylori* strains. It was demonstrated that heterologous expression of HP_P120453 in *Escherichia coli* cells led to cell growth arrest. We also showed that the toxicity of HP_P120453 was neutralised by co-expression with HP_P120452. Our data also suggest that HP_P120453 has a bacteriostatic rather than a bactericidal effect on *E. coli* cells. It is therefore concluded that HP_P120453 is a toxin and HP_P120452 the cognate antitoxin of an ICE-encoded TA system present in some *H. pylori* strains.

S5/P8

Exploiting genomics to dissect pathogenic factors of *Lawsonia intracellularis*

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Lawsonia intracellularis (LI) is a gram-negative obligate intracellular bacterium and the aetiological agent of proliferative enteropathy (PE), a common intestinal disease which affects many mammalian species, most notably pigs. The disease is characterized by the thickening of the intestinal mucosal lining and lesions in the small and large intestine. The severity of PE ranges from a mild, chronic form, often observed in grower pigs to a severe, acute form in young pigs. The complete genome of LI of 1.7MB has been fully sequenced and is composed of a circular chromosome of 1.4Mb and 3 plasmids of 27, 39 and 194Kbp. Utilizing next generation sequencing we were able to determine complete genomic sequences of several LI strain directly from cell culture samples and a similar approach is currently employed for the sequencing of LI directly from faecal samples of infected pigs. Comparative genome analysis of high and low pathogenic LI strains identified sequence and length variation in a gene encoding for a putative protein. Immunohistochemical analysis revealed that the protein is associated with the bacterial surface and expressed during early stages of infection in the intestinal crypt cells. Ongoing work involves further characterisation of the structure and function of the protein and its role in the pathogenesis of PE.

S5/P9

Assessing Phenotypic Variation of Pseudomonas aeruginosa Small Colony Variants.

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Whilst Pseudomonas aeruginosa is a well-documented nosocomial Whilst Pseudomonas aeruginosa is a well-documented nosocomial opportunistic pathogen, the bacterial small colony variant (SCV) phenotype is relatively poorly defined compared with Staphylococcus aureus, yet their significantly altered metabolic and energetic pathways complicate clinical diagnosis. Recognising and understanding the basis of these phenotypic differences has the potential to assist in more accurate diagnosis of infection in, for example chronic wounds, where these morphotypes can persist and cause recurrent infection[1,2].

Using two SCV morphotypes and a revertant strain of P. aeruginosa ATCC9027 this study demonstrates that the SCV and revertant have elevated tolerance to a number of antibiotics commonly used to treat wound infection. No differential aggregation or increased capacity to form biofilm was observed for any of the SCV or revertant phenotypes. However, scvB, scvG and scvB-Revertant all have an increased propensity to attach to and invade human keratinocytes when compared to the wild-type. Having established a set of unique, conserved phenotypic changes for the SCV and revertant morphotypes, this study aims in the future to identify the genetic changes underpinning these differences.

S5/P10

Biophysical characteristics and activities of lymphostatin: a multitalented inhibitor of lymphocyte function from attaching & effacing *Escherichia coli*

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Most non-O157 serotypes of Shiga toxin-producing *Escherichia coli* express a 365kDa protein called lymphostatin that promotes intestinal colonisation in cattle. Lymphostatin has been demonstrated to inhibit mitogen-stimulated proliferation of lymphocytes and bacterial adherence to epithelial cells, as well as being a Type 3 secreted effector protein. Although

lymphostatin has homology to large clostridial toxins at the N-terminus, including a glycosyltransferase domain, relatively little is known about its mode of action, with progress hampered by a lack of reliable assays and reagents. Here we describe the generation and characterisation of recombinant full-length lymphostatin, stably cloned and expressed from the prototype enteropathogenic *E. coli* strain E2348/69. Biophysical characterisation of the purified protein reveals a monomeric full-length protein of the expected size. Partial tryptic digestion suggests at least 3 possible structural domains, and negative stain electron microscopy has generated a 25Å resolution shell. Further, the recombinant protein inhibits the mitogen-stimulated proliferation of bovine T cells in the femtomolar range, weakly inhibits B cell proliferation, and appears inactive against NK cells. Tryptophan fluorescence assays indicate that lymphostatin binds UDP-N-acetylglucosamine with micromolar affinity, and mutation of a critical DXD motif within the putative glycosyltransferase domain ablates its activity. Elucidation of molecular signalling events influenced by lymphostatin during infection and identification of its targets will provide further insight into how this large factor influences the host response in EPEC and EHEC infection, particularly in the context of the adaptive immune response, and may help to elucidate pathways controlling lymphocyte proliferation.

S5/P11

Analysis of the entry and persistence of Salmonella enterica in the lymphatic system of cattle

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Salmonella enterica poses a substantial threat to animal health and food safety. Cattle are a key reservoir of S. enterica serotypes that cause non-typhoidal salmonellosis in humans and infections are often acquired via consumption of ground beef contaminated with infected peripheral lymph nodes. S. enterica serotypes can also cause acute enteritis and systemic typhoid-like disease in cattle, thereby exerting a significant burden on bovine welfare and productivity. Existing vaccines confer limited serotype-specific protection and a need exists to better understand the host and bacterial factors involved in pathogenesis and protection to inform the design of new vaccines and other intervention strategies. We sought to identify and characterise the host cells harbouring S. enterica in the bovine gut and lymphatic system. By infecting cattle with strains expressing green fluorescent protein and using flow cytometry we have been able to isolate infected bovine cells, determine their phenotype by analysis of cell surface marker expression, and compare tropism in the intestine and draining and peripheral lymph nodes. We also sought to identify bacterial genes required for persistence in the bovine lymphatic system using transposon-directed insertion-site sequencing (TraDIS), which involves massively-parallel sequencing of transposon-flanking regions to simultaneously assign identity and phenotype to bacterial mutants. We previously used TraDIS to assign roles for 2721 S. Typhimurium genes in colonisation of the bovine intestine. Retrospective analysis of the same mutant library but recovered from mesenteric lymph nodes draining the distal ileum in the same animals has identified genes putatively required for survival in the bovine lymphatics.

S5/P12

Developing *Caenorhabditis elegans* and its aversive behaviours as a biosensor for bacterial pathogenicity.

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In order to combat the rise of antibiotic resistant bacteria, it is essential that methods are developed which allow mechanisms of infection to be investigated. One way is through the use of invertebrate models, for example the nematode *Caenorhabditis elegans*. *C. elegans* has bacteria as its natural diet and has been shown to exhibit aversive learned behaviour in response to pathogenic bacteria. We have utilised *C. elegans* to examine aversive behaviour towards various strains of *Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa*. We observed food aversion levels and reproductive outputs (numbers of eggs and larvae produced) over 48 hours of exposure to these bacteria and compared these to *C. elegans* killing assays. This identified three strains, two *P. aeruginosa* and one *K. pneumoniae* as particularly pathogenic because they exhibited significantly high levels of food aversion ($P<0.0001$ for the *P. aeruginosa* strains, $P<0.05$ for the *K. pneumoniae*) and led to faster *C. elegans* killing relative to other strains tested. To further investigate this food aversion behaviour in response to bacterial pathogens we investigated the role of biogenic amines, which act as neurotransmitters in *C. elegans*, in controlling food aversion behaviour. We observed that serotonin deficient *C. elegans* have a reduced aversive pathogenic response, whereas octopamine deficient animals exhibited an enhanced aversive response, suggesting that octopamine is important in suppression of this aversive response. Identifying *C. elegans* factors involved in this behavioural response will enable us to elucidate pathways controlling *C. elegans* behaviour towards pathogenic bacteria.

S5/P13

T4-like environmental bacteriophages that activate ToxIN$_{Pa}$-mediated "altruistic suicide" in abortive infection

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Abortive infection is an anti-phage mechanism in which a virally-infected bacterium initiates its own premature death and reduces the production of viral progeny, protecting clonal siblings in the bacterial population by "altruistic suicide". Abortive infection can be mediated by Type III toxin-antitoxin called ToxIN$_{Pa}$ where the toxin is an endoribonuclease and the antitoxin is an RNA that inhibits the toxicity of the toxin by forming a heterohexameric complex with the toxin prior to activation. Upon specific phage infection, ToxIN$_{Pa}$ is "activated" and the toxin is released to cause lethality. However it is still unknown why only certain phages are able to activate ToxIN$_{Pa}$. To address this issue we introduced ToxIN$_{Pa}$ into *Serratia* sp. ATCC 39006 then isolated *Serratia* phages and tested if they could activate ToxIN$_{Pa}$. We have isolated three T4-like phages from a treated sewage outflow point into the river Cam in Cambridge, each of them isolated a year apart. These phages are susceptible to ToxIN$_{Pa}$-mediated abortive infection and produced spontaneous mutants that escape ToxIN$_{Pa}$ by multiple routes. The latest isolate is ΦCBH8, a dsDNA phage with a genome size of 171,175 b.p. By comparing the complete genome sequences of wild type ΦCBH8 and escape mutants we have shown that escapes can arise by mutating the *asiA* gene, or by deleting a large region of DNA with sizes ranging from 6.5kb to 10kb in different mutants. Analysis of these escape mutants may help uncover the nature of the phage product(s) responsible for the activation of ToxIN$_{Pa}$.

S5/P14

The glyoxylate shunt in *Pseudomonas aeruginosa*: first insights

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Pseudomonas aeruginosa colonizes the airways of patients with cystic fibrosis (CF). It is a very adaptable bacterium that can utilize a wide range of compounds as carbon sources. *P. aeruginosa* infections often cause serious complications - infections that are very difficult to resolve through conventional antibiotic therapy. It is imperative to develop new anti-pseudomonal strategies. In this regard, targeting the glyoxylate shunt is a promising approach. The glyoxylate shunt is a pathway that enables cells to grow on even the most simple carbon sources. It bypasses the oxidative decarboxylation steps of the TCA cycle, thereby re-routing carbon atoms towards gluconeogenesis. In *E. coli*, the first enzyme of this shunt, isocitrate lyase (ICL), has to compete for the isocitrate with isocitrate dehydrogenase (ICD). Normally, almost all of the isocitrate is fluxed around the TCA cycle because the K_M of ICL for isocitrate is much higher than the K_M of ICD. In order to achieve flux through the shunt, the ICD needs to be inhibited. This is accomplished through phosphorylation by AceK. However, it is more complex in *P. aeruginosa* because three enzymes compete for isocitrate. These are ICL, ICD and another isozyme of isocitrate dehydrogenase, IDH. I have been characterizing the structure, kinetics and regulatory properties of these enzymes, in an effort to understand how flux is redirected through the glyoxylate shunt in *P. aeruginosa*. To do this I have been collecting structural and kinetic data on the purified enzymes, as well as characterizing the expression profile(s) in different growth conditions.

S5/P15

Studies on the intracellular life of the melioidosis pathogen *Burkholderia pseudomallei*

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Melioidosis, caused by the environmental Gram negative bacillus *Burkholderia pseudomallei*, is an emerging infectious disease of animals and humans, commonly presented as pneumonia or abscess formation in internal organs and also soft tissues that can progress to septic shock. As a facultative intracellular pathogen, *B. pseudomallei* has the ability to enter the host cell, and escapes from the phagosome. Once in the cytoplasm, the pathogen proliferates and moves inter- and intracellularly via BimA-dependent actin-based motility (ABM). In the cases of *Listeria monocytogenes* and *Shigella flexneri*, the bacterial proteins required for ABM (ActA and IcsA respectively) are also involved in avoidance of host cell autophagy, thereby allowing the bacteria to persist and replicate in infected cells. In BALB/C bone marrow derived macrophages (BMDM), a *B. pseudomallei bimA* mutant displays impaired intracellular survival compared to wild-type bacteria at late time points post-infection. We speculated that BimA mediates escape from autophagy. However our studies, including LC3-conversion assays, bacterial co-localisation studies and assessment of p62 activation, failed to demonstrate a role for autophagy in clearance of the *bimA* mutant from infected BMDM. Hence, we hypothesise that ABM mediates escape of *B. pseudomallei* from macrophage intracellular killing mechanisms, through an as yet undefined mechanism.

S5/P16

The intracellular phase of *Streptococcus pneumoniae* as a novel mechanism of immune evasion during monoclonal bacteraemia

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Streptococcus pneumoniae, an obligate commensal pathogen of humans, causes invasive diseases associated with bacteraemia. In an experimental mouse model, bacteraemia was observed to originate from clonal expansion of a single bacterial cell (Gerlini, PLoS Pathogens 2014).

To investigate the origins of this single cell bottleneck, CD1 mice were inoculated intravenously and tissues were analysed at the early stages of infection. Pneumococci were found predominantly within the spleen, the major clearance organ in non-immune mice. Confocal microscopy of spleen sections, simultaneously infected with GFP and RFP tagged bacteria, showed numerous foci of infection, each one shown to have originated from the replication of a single pneumococcus. Further, these foci were localised prevalently within metallophilic macrophages where bacteria persisted and replicated over time, while in the marginal zone macrophages pneumococci were rapidly cleared.

These data highlight the crucial role of an intracellular phase as a newly identified immune evasion strategy in the pathogenesis of pneumococcal bacteraemia. Why the ensuing bacteraemia is founded by only one of the numerous single cell foci found in splenic metallophilic macrophages and how this bacteraemia is sustained over many hours is now the major challenge for future studies.

S5/P17

Promotion of filamentous growth in *Candida albicans* by *Pseudomonas aeruginosa* secretions: Identification of novel players in cross kingdom communication.

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Pseudomonas aeruginosa (PA) and *Candida albicans* (CA) are commonly found together *in vivo* with the antagonistic or synergistic nature of their interaction still under debate. We have developed an assay to study the effect of PA secretions on CA growth. Our data suggest that wild type (wt) PA secretions induce filamentation of CA, a key virulence attribute of this fungus. Using appropriate PA mutants, we have shown that this observation is not due to the production of rhamnolipids, exotoxin A, or dependent on the virulence regulator *ToxR*. Interestingly, promotion of filamentous growth does not take place when using supernatants from a PAO1 mutant having a spontaneous 58-Kb chromosomal deletion (PAO1-LΔ58Kb), indicating that the gene(s) responsible for promoting hyphal growth in CA is/are encoded within this region. Both biochemical and genetic approaches are being exploited to determine the nature of the active components and mechanism of action. Activity is eliminated by heat (95°C, 10 min), showing the possibility of the hyphal-inducing agent being a protein. Using Amicon® Ultra-15 centrifugal filters to fractionate PA supernatants; the CA hyphal induction activity was retained in ultrafiltrates of molecular weights above 10 kDa, which is in agreement with preliminary SDS-PAGE analysis showing the presence of two 8– 12 kDa bands in the wt supernatants. Considering the influence of cross-kingdom communication on microbial pathogenesis and the difficulty in treating polymicrobial infections, this work presents new insights into the interactions that occur between PA and CA during co-infections in Cystic Fibrosis patients and burn/trauma victims.

S5/P18

Distinct effects of Interferon gamma and Interleukin-17A on the response of macrophage-neutrophil co-cultures to Pseudomonas aeruginosa

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P. aeruginosa (PA) causes serious infections in immunocompromised and burn patients and is especially troublesome in Cystic Fibrosis (CF). We observed a positive and a negative correlation between IFN-γ and IL-17A production by CF immune cells respectively and lung function which suggest that,

either absence of Th1 and/or over exuberant Th17 responses might contribute to a deterioration of lung function in CF. Treatment of macrophages with IFN-γ failed to alter bacterial growth or macrophage survival upon PA infection, but made the macrophages more pro-inflammatory. There was therefore the need to include neutrophils as the effector phagocytes to determine how inflammatory conditions affect PA clearance and potential for tissue damage. We established a macrophage-neutrophil co-culture model and investigated the effect of IFN-γ and IL-17A on the ability of these co-cultures to control PA growth, cause tissue damage and promote inflammation. Our data show: (1) neutrophils and macrophages collaborate in clearing PA infection; (2) IFN-γ does not promote PA clearance by macrophage-neutrophil co-cultures; (3) IL-17A has a marginal positive effect on PA clearance and (4) IFN-γ and IL-17A do not affect production of neutrophil elastase. Interestingly, (5) neutrophils promote IL-1γ production in the co-cultures and this effect is abolished in the presence of IFN-γ. These results suggest that IFN-γ might be protective in CF because of its ability to restrict IL-1β production and, consequently, reduce inflammation. Meanwhile, work is in progress to determine the mechanism behind this finding.

S5/P19

Molecular mechanisms of *Campylobacter jejuni* pathogenesis and survival in the environment

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Campylobacter jejuni is the major foodborne gastrointestinal pathogen. The wide of use of antibiotics in medicine and in animal husbandry has resulted in increased incidence of antibiotic resistance in *Campylobacter*. In this study, *C. jejuni* G1 strain was found to be more resistant to tetracycline (Tet) when compared with the reference strain NCTC11168, despite the absence of the Tet resistance gene *tet(O)* in both strains. Comparative genomics analysis revealed a remarkable difference between the sequences of the *cmeB* genes in these strains. The inner membrane protein CmeB is a component of the CmeABC multidrug efflux pump. Mutation of gene *cmeB* in the G1 strain resulted in a 32-fold reduction of resistance to Tet and increased sensitivity to a number of other antibiotics, confirming the predicted role of CmeB. Transfer of the pTet plasmid from *C. jejuni* 81-176 to the G1 strain increased the level of Tet resistance above that of the former, suggesting that CmeB of strain G1 has a higher potency to excrete this drug than that of the orthologues in strains 81-176 and 11168. Preliminary experiments using *Acanthamoeba polyphaga* as a model organism also suggested a possible role of the CmeB protein in invasion of, and survival within these host cells, which may be important for bacterial protection in adverse environmental conditions. In addition, site-directed mutagenesis of a capsular polysaccharide-related gene *kpsM* led to decreased ability of strain 81-176 to invade *A. polyphaga*, suggesting a role of the capsule in this process.

S5/P20

IL-10-producing lymphocytes in the immune response to Helicobacter pylori infection

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In the human gastric mucosa, a host inflammatory response stimulated by the bacterial pathogen *H. pylori* can lead to peptic ulceration and gastric adenocarcinoma. Vacuolating cytotoxin A (VacA) promotes the differentiation of regulatory T cells (Tregs), which suppress inflammation through interleukin-10 (IL-10) production, and allow persistent *H. pylori* colonisation. An increased risk of disease is observed in those with an inadequate Treg response, but the role of regulatory B cells (Bregs) is yet unknown. This work aimed to quantify IL-10-producing Breg and Treg populations in an infected animal model, and in human blood.

Splenic lymphocytes were isolated from infected and uninfected mice. Human peripheral blood mononuclear cells (PBMCs) were isolated from donated samples. These were stimulated with mitogens *in vitro*, before being stained with fluorochrome-conjugated antibodies for flow cytometry analysis.

In the mouse spleen, B-cells were the main lymphocyte source of IL-10 (~70%). The size of the IL-10-producing B-cell population was not influenced by infection, however $CD4^+$ T-cell frequencies amongst the $IL\text{-}10^+$ lymphocytes increased significantly (mean±SEM%: infected 16.9 ± 1.4, uninfected 12.1 ± 0.9, n=4 per group, $p<0.05$). Frequencies of both $CD5^+CD1d^{hi}$ Bregs and $Foxp3^+$ Tregs amongst the IL-10-producing lymphocytes also increased after infection. In human PBMCs, half of the $IL\text{-}10^+$ lymphocytes were $CD4^+$ T-cells. While B-cells were much less abundant than $CD4^+$ T-cells, they accounted for a third of the IL-10 producing-lymphocytes.

Data from both species revealed B-cells to be an important source of IL-10. We are now investigating the potential role of these cells in *H. pylori*-mediated disease.

S5/P21

Identification of platelet receptors for Streptococcus gallolyticus

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Streptococcus gallolyticus is a commensal occurring at 2.5 to 15% in the healthy human gastrointestinal tract. It is a causative agent of infective endocarditis, an infection of the endocardial surface of the heart. More notably, a strong association between the presence of colonic cancerous abnormalities and the isolation of S. gallolyticus strains from infective endocarditis

lesions has been described. The reason for this correlation is unknown. To date there have been no studies on the interaction of *S. gallolyticus* with platelets. In this study, the aim is to identify platelet receptors for *S. gallolyticus*.

Initial results indicate that the adherence of *S.gallolyticus* to platelets varies in a strain specific manner. Antibodies and recombinant protein receptors to platelet surface receptors GP11b, CD36, TLR2, TLR4, P-Selectin 2 were used as inhibitors of platelet adherence by *S. gallolyticus*. Common platelet receptors have been identified that promote platelet adherence by S. gallolyticus. The adherence phenotype of the bacterium also appears to have a growth phase dependency.

Identification of platelet receptors for *S.gallolyticus* strains offers the potential to develop antimicrobial therapeutics that may advance treatment of *S. gallolyticus* Infective Endocarditi.

S5/P22

The Evaluation of a Peptide Based Delivery System to Improve the Effectiveness of Moenomycin A Against Both Encapsulated and De-capsulated *Streptococcus equi* subsp. *equi* ATCC4047.

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Streptococcus equi subsp. *equi* ATCC4047 is a capsulated Gram positive bacteria with numerous virulence factors enabling it to avoid host immune systems when causing the disease; Strangles, in horses. The disease is arguably one of the worst known infectious diseases affecting horses in the world, with no viable treatment method available and extreme quarantine and cleaning approaches being the only means of preventing further infection.

Moenomycin A is a known antimicrobial which targets the active site of the GT51 glycosyltransferase protein. GT51 is localised adjacent to the bacterial cell membrane and its inhibition prevents the development of the important cell wall polymer peptidoglycan. Moenomycin A is known to be relatively ineffective against Gram negative bacteria due to poor delivery through the outer lipid membrane. This study tests the effectiveness of a peptide based delivery system which is designed to aid Moenomycin A in passing through the hyaluronic capsule of *Streptococcus equi* to the GT51 protein.

This ongoing study looks at removing the capsule of *Streptococcus equi* with Hyaluronidase and performing Minimum Inhibitory Concentration and Minimum Bactericidal Concentration tests with Moenomycin A as well as Moenomycin A coupled to delivery peptides. The effectiveness of the delivery system will be measured by evaluating the Minimum Inhibitory Concentration of encapsulated and de-capsulated *Streptococcus equi.* This will determine the effectiveness of the delivery system through hyaluronic acid and whether removal of the capsule would increase sensitivity to the Moenomycin A, in order to provide potential better treatment methods.

S5/P23

Identification of attenuated *Salmonella* pullorum mutant s and corresponding genes in the chicken embryo infection model by PCR signature -tagged mutagensis

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Salmonella Pullorum could be transmitted to offspring chilken going through egg hatching. Using PCR signature-tagged mutagensis, 9 genes of Salmonella Pullorum were identified in the chicken embryo infection model. They involved in invasion, multiplication, metabolism, defense. The competition index in vivo and in vitro and virulence evaluation to chicken embryos of 9 mutant strains clarified that they were attenuated.

S5/P24

The surface proteome of Gram-negative bacterial pathogens

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The emergence of multi- and pan-drug resistant microorganisms highlights the need for vaccines as an alternative strategy to fight enterobacterial pathogens. The bacterial cell surface is the first point of contact between pathogens and host, and is therefore also one of the most complex and variable parts of the bacterial proteome. Subtle differences can lead to changes in tissue specificity of bacterial pathogens, and our work has focused on assessing the diversity of different bacterial cell surface proteins and secretion systems, and the chaperones necessary for their correct folding (Celik et al. 2012 PLoS ONE, Webb et al. 2012 Trends Microbiol., Dunstan et al. 2013 PLoS Pathogens, Heinz et al. 2014 Front Microbiol., Heinz et al. 2015 Genome Biol Evol., Heinz et al. in prep., Stubenrauch et al. in prep.). We are now applying the gained insights on the diversity and evolution of these protein families to assess the fine-scale diversity of several of the most important pathogens, with a focus on the Genera of Salmonella and Klebsiella. A better understanding of the outer membrane protein diversity beyond presence/absence, focusing at the evolution as well as domain/sequence diversity in these which include human pathogens, animal pathogens and commensal organisms will give us crucial insights regarding tissue specificity, immune system evasion and how organisms with different success strategies modify their surface to evolve into highly successful human pathogens.

S5/P25

Understanding phenotypic diversity in chronic wounds in populations of *Pseudomonas aeruginosa*

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Chronic leg and foot ulcers are a recurrent complication of diabetes. Polymicrobial infections are frequently present in diabetic ulcers often resulting in severe antibiotic-resistant bone infections (osteomyelitis) that ultimately lead to amputation. Previous studies of the common opportunistic pathogen *P. aeruginosa* in chronic lung infections suggest that considerable phenotypic diversity arises during the course of infection, but it is not known whether this is also the case in chronic wounds such as diabetic ulcers.

We phenotypically analysed 120 individual *P. aeruginosa* isolates taken from the bone, soft tissue and blood of a patient with a diabetic ulcer that ultimately resulted in leg amputation due to the intractability of the infection. We focused on phenotypes that have traditionally been associated with *P. aeruginosa* pathogenicity in wounds. All 120 isolates produced the quorum sensing signal C4-HSL, but none of them produced pyocyanin, proteases or 3O-C12-HSL. However, colony morphology on Congo red agar and siderophore production revealed two distinct phenotypic groups. All 40 blood isolates and 16 of the soft tissue isolates formed red and wrinkly colonies with low levels of pyoverdine and high levels of pyochelin. While all 40 bone isolates and 24 soft tissue isolates formed white, smooth colonies with moderate levels of both pyoverdine and pyochelin. Our data suggests that phenotypic diversity in *P. aeruginosa* populations is not as pronounced in chronic wounds as it is in CF infections, and that virulence factors commonly associated with infection are not required for the long-term colonisation of chronic diabetic wounds.

S5/P26

Escherichia coli platelet adherence: The role of LPS and platelet receptors

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Sepsis is an inflammatory response to bloodstream infections with high mortality rates. *E.coli* is the most frequent gram negative causative organism of severe sepsis. Bacterial-platelet interactions are vital in the pathogenesis of sepsis. My research has shown that membrane receptors on platelets recognise Lipopolysaccharide (LPS) and directly bind to it.

This study demonstrates that CD36, TLR2 and TLR4/MD2 are platelet receptors for the direct adherence of *E.coli* LPS to platelets. LPS comprises three main components; the O-antigen, a core polysaccharide and the lipid A region. Our work reveals that the minimum LPS components required for *E.coli* binding to platelets and CD36 must contain the Lipid A and inner core regions of LPS.

In addition to this, analysis of the adherence of epidemic strains of pathogenic antibiotic resistant strains of *E. coli* to platelets and recombinant platelet receptors showed that the O-antigen portion of LPS may conceal LPS from recognition by these receptors as these strains bind significantly less to CD36 compared to an *E. coli* strain that does not express an O-antigen.

Interestingly, murine CD36 has a significantly reduced affinity for *E.coli* compared to human CD36. This may help to explain why the human and mouse immune response to *E.coli* infection is different and this contributes to the failure of anti-sepsis drugs developed in mouse models of infection, like Eritoran in human clinical trials.

S5/P27

Role of vacuolating cytotoxin A forms in *Helicobacter pylori*-mediated immunomodulation

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Helicobacter pylori is the major cause of peptic ulceration and gastric cancer. The vast majority of infections are asymptomatic however, and the ability of *H. pylori* to modulate host immunity may provide some protection against allergies and autoimmune diseases. Recent studies suggest that the major virulence factor vacuolating cytotoxin A (VacA) exerts tolerogenic effects on dendritic cells (DCs), leading to expansion of regulatory T cells. The *vacA* gene is present in all strains but is polymorphic, with two allelic variants existing in its signal (s1/s2), intermediate (i1/i2) and mid (m1/m2) regions. Although expression of the s1/i1/m1 form of VacA is associated with disease risk, the effect of different VacA forms on *H. pylori*-mediated immunomodulation is poorly understood.

Here we show that strains expressing the s1/i1 form of VacA stimulated significantly stronger IL-10 responses in the spleen of infected mice (1980 ± 308 pg/ml vs. 1295 ± 78 pg/ml, $p < 0.05$) and the gastric mucosa of infected patients (4.25 ± 0.58 pg/ml vs. 1.68 ± 0.33 pg/ml, $p < 0.01$) compared to s2/i2 strains. *In vitro*, both human and murine DCs were found to secrete significantly higher levels of IL-10 in response to s1/i1 strains. LPS-induced DC maturation was significantly inhibited by an s1/i1 strain (≈50% inhibition), but inhibition by an isogenic s2/i1 mutant was less effective (≈30%).

These results demonstrate that s1/i1 forms of VacA have stronger immunomodulatory activity. Further studies are required to characterise the role of different VacA forms in protection against inflammatory and autoimmune extra-gastric diseases.

S5/P28

Investigation of Pathogenicity and inflammatory role of selected *Cronobacter* isolates from French outbreak 1994 in nomalegnant Human intestinal epithelia (H4)cell line

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The genus *Cronobacter* has recently involved in many outbreaks in neonatal intensive care units worldwide, with illness features of meningitis, bacteraemia, and necrotizing enterocolitis (NEC) and high mortality rate. Although this genus was deeply investigated *invitro* with different cell lines, until now, few researches have been focused on the interaction with H4 cells. Three *C. sakazakii* isolates form French outbreak 1994 which were involved in NEC type III, meningitis and septicaemia were chosen in this study. Bacterial isolates were cu-cultured with the H4 cells to investigate their ability to attach and invade H4 cells. Cytotoxicity of these strains to H4 cells was also investigated and cells viability determined using trypan blue method. Moreover the inflammatory response of H4 cells was investigated using Human IL-8 ELISA Kit. Our finding indicated that the tested strains were able to attach and invade H4 cells, and the NEC III isolate was the highest. Cytotoxicity results presented as a folds of the blank, and ranged from 1.34 showed by NEC III strain to 2.41 showed by meningitic strain. Inflammatory response to the selected strains was extremely high compared with the controls. The produced IL-8 was 4424 pg/ml, 3145 pg/ml and 1628 by strains responsible for Meningitis, NEC III and Septicaemia respectively. To our best knowledge, this is the first research of these strains with H4 cells. However our results strongly support the previous finding of the role of this genus in neonatal infections mainly the inflammatory and NEC complications.

S5/P29

Genotyping of potentially pathogenic *Acanthamoeba* strain isolated from an infected wound patient in Colchester

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Free Living Amoebae (FLA) are widely distributed in the environment. They have been isolated from soil, water, air and other environmental sources. Some species of *Acanthamoeba* cause central nervous system (CNS) infections; Granulomatous Amoebic encephalitis (GAE) in immunocompromised patients. One of the entry routes for *Acanthamoeba* is through skin lesions, then to the brain via haematogenous spread. However, the role of *Acanthamoeba* in wound infections is not very well understood. In this study, 140 wound swabs were collected to check for the presence of *Acanthamoeba spp*. All samples were cultured on non-nutrient agar plates seeded with *Escherichia coli*, incubated at 30 °C, and examined periodically. One sample was positive for the presence of *Acanthamoeba spp*. DNA was extracted; and PCR was applied to amplify *Acanthamoeba*-specific amplimer S1 (ASA.S1). Identification of the isolate was based on PCR-sequencing of the Diagnostic Fragment 3 (DF3) to identify strains at the genotype level. Sequencing of the highly variable DF3 region of the 18S rRNA gene for the sample showed that this isolate belongs to genotype T4 and subtype A. This study is the first to look at the genomic characterisation of *Acanthamoeba* strain in wounds and the first report of *Acanthamoeba* in Colchester.

S5/P30

Evasion of innate immune recognition by Uropathogenic *Escherichia coli* is modulated via the composition of its outer membranes

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Uropathogenic *Escherichia coli* (UPEC) is a major cause of urinary tract infections worldwide. Genetically, UPEC differs from other *E. coli* pathotypes through virulence factors that facilitate its microbial colonisation of the host urinary tract. The innate defence system of the host uroepithelium recognises pathogen-associated molecular patterns (PAMPs) via toll-like receptors (TLRs) that activate the pro-inflammatory signalling pathways involving the transcription factor NF-κB. Analysis of the innate immune response in the immortalised bladder epithelial RT4 cell line, stably transfected with a NF-κB reporter, suggested that the bacterial flagellar subunit, flagellin, was key in inducing activation the innate immune system via NF-κB. Using a panel of clinical UPEC isolates it was shown however, that not all motile UPEC strains induced the innate immune system defences, although flagellins isolated from such strains did. This has led to the question: how does *E. coli* modulate flagellin recognition?

To address this question activation of RT4-NF-κB signalling using flagellin in combination with outer membrane components isolated from clinical UPEC isolates was explored. Outer membranes used were isolated from wild type UPEC isolates or mutants with altered assembly status of the flagellum and/or fimbriae. In our assays we have identified UPEC isolates whose outer membranes induce intermediate responses compared to nonreactive outer membranes and flagellin controls.

This suggests that there is a selective pressure for *E. coli* in the urinary tract to modulate its outer membrane composition to reduce its recognition by the innate defences of the urothelium.

S5/P31

Surface exposed loops of the OmpA2 Outer Membrane Protein of *P. gingivalis* are key to Host-Pathogen Interactions.

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Evasion of the host is an important virulence factor for disease progression, and as part of pathogenic features of the keystone periodontal pathogen, *Porphyromonas gingivalis*, the ability to invade epithelial cells is crucial for host evasion- i.e. inhabiting an immune-privileged niche. As part of the invasion process surface associated proteins, are central. We have identified the outer membrane protein encoding *ompA* genes as being potentially important in invasion, since it is part of a signature set of genes identified as differentially regulated in an invasive subtype of the *P. gingivalis* population. The aim of this study is to investigate the role of *ompA* and other signature set genes in invasion of oral epithelial cells and in biofilm formation.

Strains lacking the *ompA1-2* operon and individual *ompA* genes have been created, alongside a *ompH1-2* (*skp*) chaperone mutant that lacks a range of outer membrane proteins including OmpA complex. Knockout of the *ompA1-2* genes almost completely abrogates attachment to and invasion of epithelial cells and formation of biofilm in vitro compared to wild-type, whereas the *ompA2* mutant shows a two-fold reduction. The Δ*ompH* mutant shows similar abrogation of adherence and invasion. To investigate direct OmpA-human interactions, the protein structure was modelled, and proposed surface-exposed OmpA peptides synthesised reduced interactions with epithelial cells. Furthermore, these peptides in isolation are able to direct interaction of inert agarose beads with host cells. These results illustrate a role for surface loops of the OmpA protein in the adherence and invasion of host cells.

S5/P32

Characterization of human B-cell derived monoclonal anti-*Candida* antibodies with potential use as diagnostics, therapeutics and tools in basic sciences

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Monoclonal antibodies (mAbs) have become essential tools for diagnosing and treating a wide range of diseases. Surprisingly, the outstanding potential offered by mAbs has not been exploited by the field of infectious diseases. Our laboratory generated a panel of recombinant human anti *Candida* mAbs using a novel single B cell antibody technology that were raised against (a) Hyr1 – cell wall proteins and (b) whole cell walls. Here we report on characterization of these mAbs in terms of their binding profiles and bio-reactivity.

The *Candida albicans* cell wall is a two layered structure. The inner layer is composed of β-glucans and chitin, and the outer layer comprised of a fibrillar layer composed of glycosylated mannoproteins post-translational modified with *O*- and *N*-linked mannosides. We examined changes in mAb binding to *C. albicans* cell wall mutants with reduced phosphomannan and mannan layers. We also compared mAb binding to *C. albicans* cells before and after enzymatic treatments which removed proteins, β-glucans and high mannose N-glycans from cell wall glycoproteins. Binding of mAbs to other pathogenic fungi was also examined. The antibodies strongly opsonised *Candida* cells, influencing the behaviour of macrophages towards *Candida* targets and providing protection against systemic inoculation of *Candida* in a mouse model.

Our data demonstrate that the panel of mAbs tested were all *Candida*-specific. mAbs bound to different cell wall components recognizing multiple protein and carbohydrate epitopes. Current investigations are identifying protective epitopes which could serve as novel targets in vaccine development and contribute to designing powerful diagnostic and immunotherapeutic approaches.

S5/P33

Investigation of phase variation of Opa proteins in Neisseria meningitidis during persistent carriage.

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Neisseria meningitidis is one of the main causes of bacterial meningitis and septicemia. It also colonizes the upper respiratory tract of humans asymptomatically as a normal commensal. Phase variation (PV) in the surface antigens is proposed as an effective mechanism to enable these bacteria to adapt and persist in the human host. Three to four Opa proteins are expressed on the outer surface of meningococcal cells playing an important role in the pathogenicity by mediating the adhesion to and invasion of human cells. These proteins are encoded by three/ four loci and each locus is phase variable due to pentameric tracts within the coding region. The phase variability in Opa proteins was investigated in meningococcal isolates from 19 carriers and time points representing up to six months of asymptomatic carriage. Changes in repeat tracts were analyzed by GeneScan, and a high frequency of PV was observed in at least two loci with a rate of 0.06 mutations /gene/ month during colonization. The expression state of Opa was confirmed

by Western blotting indicating expression of a limited number of Opa variants. Around 70 % of the isolates expressed only one Opa and none simultaneously expressed four Opa. Intergenic and intragenic recombination was detected in two carriers, leading to new *opa* alleles. These results revealed that persistent carriage was correlated with a high rate of variation and switching between different Opa variants with stable expression of one or more alleles that may maintain Opa-mediated adhesion.

S5/P34

Manipulating recycling pathways to alter the surface of infected cells.

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Many bacterial pathogens manipulate infected cells by injecting proteins to target small GTPases. Small GTPases coordinate a wide range of cellular processes including cytoskeletal rearrangement, membrane trafficking and nucleocytoplasmic transport. We have identified an Arf6/Rab35 small GTPase signaling platform that is targeted by an injected effector protein from enterohaemorrhagic *Escherichia coli* (EHEC) and related pathogens. The Arf6/Rab35 signaling hub is increasingly being recognised as a key regulator of endocytic recycling and indeed we have found EHEC alters recycling endosome function during infection. Disrupting recycling endosomes results in the reduction of a subset of proteins on the surface of an EHEC infected cell. Proteins with reduced surface localization have roles in solute uptake, immune cell activation, regulating cell survival and maintaining cell-cell contacts, all of which contribute to the infection strategy of these pathogens.

S5/P35

Ammonia production is important for staphylococcal resistance to sapienic acid, an antimicrobials lipid produced by human skin

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Staphylococcus aureus and *Staphylococcus epidermidis* are leading causes of hospital-acquired infections. These infections are typically seeded from the patient's flora, as such, understanding how these bacteria survive on skin is key to developing methods to prevent staphylococcal infections. Low levels of sapienic acid on skin is linked to increased staphylococcal colonisation, suggesting sapienic acid is an innate host defence against staphylococci. The minimum inhibitory concentration of sapienic acid was higher in *S. epidermidis* than in *S. aureus*, which is consistent with higher levels of *S. epidermidis* found on healthy skin than *S. aureus*. Using RNA Seq, the transcriptomic responses of *S. aureus* and *S. epidermidis* to sapienic acid were compared. These data were used to search for common candidate genes regulated in response to sapienic acid in staphylococci. A novel role for ammonia production in sapienic acid resistance was discovered. Sapienic acid is known to cause a decrease in the internal pH of staphylococci which we predict Could be countered by ammonia production. Further studies will investigate novel genes highlighted from our comparative transcriptomics data of sapienic acid resistance.

S5/P36

The interaction between *Pseudomonas aeruginosa* with C-Type Lectins

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Colonization and persistent lung infection by *Pseudomonas aeruginosa* (PA) are important causes of mortality and morbidity in cystic fibrosis patients. PA persists inside the lungs by forming drug-resistant biofilms. These biofilms are enclosed in an extracellular polymeric substance (EPS) which functions as a sticky matrix that protects bacteria from harsh environmental agents. The polysaccharide synthesis locus (*psl*) and *pel* are important EPS components that promote biofilm formation and cell aggregation. No information is available on immune receptors specifically involved in biofilm recognition. We hypothesize that C-type lectin receptors such as mannose receptor (MR) and DC-SIGN might play a role in the recognition of PA by the immune system through binding to EPS. Our results show that MR binds PA biofilms through the CTLD4-7 region and that this binding is psl-dependent. We also found that DC-SIGN binds to PA biofilms and extracted EPS significantly better than MR CTLD4-7. Unexpectedly DC-SIGN also binds whole PA planktonic cells. Future work will focus on the identification of DC-SIGN ligands in planktonic PA and determine how biofilm components psl and pel modulate recognition of PA biofilms by human myeloid cells expressing DC-SIGN and/or MR. The findings will reveal how EPS influences the recognition of PA by immune cells which might lead to the discovery of novel therapeutic targets.

S5/P37

Identification and characterization of fibrinolytic extracellular protease from *Microbacterium* sp.

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The increased fibrinolysis and disruption of cardiovascular system homeostasis caused by microbial proteases can facilitate the colonization of the host organism by pathogens.

We have observed fibrinolytic activity of culture media after *Microbacterium* sp. strain JK6B cultivation and identified extracellular protease responsible for such a property. Mature form of microbacterial fibrinolytic protease A (MfpA) was purified from culture medium, subjected to N-terminal sequencing and further biochemical characterization. The *mfpA* gene was cloned, sequenced and heterologously expressed in *Escherichia coli*. Recombinant protein was enzymatically active.

The protease contains HExxHxxGxxH motif characteristic for M12 metalloendopeptidases family, and its activity was substantially reduced by 1,10-phenanthroline.

MfpA protease is able to degrade fibrinogen (visualized by SDS-PAGE and zymography) and fibrin clots formed *in vitro* (turbidimetric tests). Moreover the protease is active toward other protein (azocasein and azocoll) and synthetic chromogenic (S-2251 and S-2765) substrates.

Analysis of fibrinogen digestion products (by peptide sequencing and mass spectrometry) revealed substrate specificity of the enzyme. MfpA protease preferentially hydrolyzed peptide bonds preceding hydrophobic residues.

Collection of 26 *Microbacterium* strains related to JK6B was tested for fibrinolytic properties. Interestingly, among 16 isolates from human wounds, tissues, blood or hospital facilities 11 produced fibrinolytic protease with molecular weight corresponding to MfpA (zymography with fibrinogen), whereas the same was true only for 3 out of 9 environmental strains. The correlation suggests MfpA protease as a potential virulence factor.

S5/P38

In vitro analysis of *Enterococcus faecalis* gene expression under urinary tract-related stress conditions

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Urinary tract infections (UTI) are the most prevalent infections caused by enterococci with *Enterococcus faecalis* being the most common causative species. The aim of the investigation was to develop a sensitive and reproducible method for the study of gene expression in clinical isolates of E. faecalis grown in conditions mimicking the urinary tract.

Enterococcal gene expression was studied using real-time reverse transcription PCR (RT-PCR) during the mid-exponential phase of growth in cells exposed to stresses commonly encountered in the urinary tract. First, the stability of expression of three housekeeping genes (*adk, 23SrRNA* and *atp*) was evaluated on the basis of the mean-CT values under different conditions. Subsequently, *E. faecalis* growth under stress was investigated (high concentrations of urea, salts, glucose, extreme pH, and exposure to ciprofloxacin) using serine hydroxymate as a stringent response (SR)-inducing positive control. An increase in expression of *relA* (a marker of induction of the SR) was determined. All the housekeeping genes included were stably expressed under the conditions investigated and consequently were included in the normalization procedure. Next, the geometric mean of the internal control genes was used to correct the gene of interest (*relA*). The level of expression of *relA* in *E. faecalis* showed significant up-regulation under stress conditions relevant to the urinary tract. This study therefore, lends support to the use of real-time quantitative RT-PCR, with adenyl kinase, 23SrRNA and atpase as internal controls for future investigation of virulence gene expression of *E. faecalis* under stress during infection of the urinary tract.

S5/P39

Characterization of toxin-antitoxin system PemIKSp from *Staphylococcus pseudintermedius*

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Toxin-antitoxin systems (TA) are located both in plasmid and chromosomal DNA of bacteria. By the involvement in the production of persister cells and virulence of these microorganisms, TA system are the object of intensive studies. The presented research focuses on the computational and functional analysis of chromosomally encoded TA system $pemIK_{Sp}$ of *Staphylococcus pseudintermedius*, homologous to the plasmid-located $pemIK_{Sa}$ system of *Staphylococcus aureus* strain CH91[1].$pemIK_{Sp}$ operons of twenty *S. pseudintermedius* strains were sequenced and analyzed. Gene variants of $pemK_{Sp}$ toxins and $pemI_{Sp}$ antitoxins were cloned into expression vectors, and recombinant proteins were produced in *Escherichia coli* BL21(DE3). The toxins were tested for their endoribonucleolytic activity towards the phage MS2 RNA. The cross-interaction with the system elements derived from *S. aureus* was analyzed by co-expression and co-purification tests. In the sequenced operons two and four variants of $pemK_{Sp}$ toxin and *pemISp* antitoxin, respectively, were identified. Endoribonucleolytic activity for toxins was confirmed. Additionally for the full-length toxin the interaction with $PemI_{Sa}$ antitoxin assessed. In *S. pseudintermedius* strains we identified a set of variants of the $pemIK_{Sp}$ system. The genetic changes within the $pemIK_{Sp}$ locus and functional toxins strongly suggest that the change of the operone location from a plasmid to the bacterial chromosomes do not cause the loss of function. Results of the research may cast a new light on the debate on prokaryotic chromosomally

encoded TA systems concerning their potential regulatory role, which stays in opposition to claims that these are selfish genetic entities only [2].

S5/P40

Proteolytic targets of Meningococcal Neisseria Autotransporter Lipoprotein (NalP) during Pathogenesis

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Neisseria meningitidis is normally a human nasopharyngeal commensal but is also capable of causing life-threatening septicaemia and meningitis. Autotransporter (or type V-secreted) proteins are an important class of neisserial virulence factors. Eight autotransporters have been identified in meningococci: IgA1 protease, NhhA, AutA, AutB, NadA, App, MspA and NalP (also known as AspA). NalP is a phase-variably expressed serine protease which cleaves a number of cell surface proteins including itself, MspA, App, IgA1 protease, Lactoferrin-binding protein B (LbpB) and Neisserial heparin-binding protein A (NhbA). The consequences of this proteolytic activity on meningococcal pathogenesis are yet to be fully determined, but have already been shown to influence the sensitivity of meningococci to killing by human whole blood and the ability of meningococci biofilm formation. To enhance our understanding of the role of NalP during meningococcal pathogenesis, we purified functional recombinant NalP passenger domain under non-denaturing conditions using immobilized nickel chromatography. The purified recombinant NalP passenger domain was shown to be proteolytically active in in vitro assays and to cleave a number of host proteins likely to play important roles in host-pathogen interactions.

S5/P41

Non-protective role of *sigB* against oxidative stress in *Listeria monocytogenes*

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Listeria monocytogenes is an important foodborne bacterial pathogen which can cause listeriosis, a life-threatening disease. The alternative sigma factor (σB) is a well-known transcriptional regulator involved in regulation of the expression of numerous stress- and virulence-related genes. However, its role is still controversial regarding oxidative stress. Some studies describe increased susceptibility for Δ*sig*B whereas others describe the opposite. In this study we aimed to clarify the role of *sig*B against oxidative stress during different stages of growth, using both a chemical stimuli (hydrogen peroxide, H_2O_2). *Listeria monocytogenes* 10403S and EGD-e, Wild Type (WT) and Δ*sig*B, were challenged with H_2O_2 in mid-exponential and stationary phase of growth and survival was assessed. In parallel, the levels of dissolved oygen (DO) were measured and the catalase activity was determined. During mid-exponential phase catalase activity was minimal and there was no major difference in survival or catalase activity between WT and Δ*sig*B. However, during stationary phase the *sigB* mutant excreted significantly higher catalase activity than the WT which was accompanied with higher resistance to H_2O_2 in both EGD-e and 10403S strains, despite both no differences in DO levels. Apparently, SigB seems to significantly increase sensitivity to oxidative stress despite its major role in general stress resistance. Furthermore, we found no role for *Sig*B in intracellular proliferation which coincides with previous work showing complete lack of sigB expression intracellularly.

Keywords: *Listeria monocytogenes*; oxidative stress; *sigB*

S5/P42

The intracellular pathogen *Salmonella* Typhimurium

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The intracellular pathogen *Salmonella* Typhimurium is a facultative anaerobe, with O2 as the preferred electron acceptor. During periods of anoxia Salmonella can switch to using nitrate in a respiratory process called denitrification. Unlike many soil bacteria, *Salmonella* undergo truncated denitrification, where nitrate is converted into nitrous oxide via sequential reduction of nitrite and nitric oxide (NO). During infection, *Salmonella* also encounters NO as part of the host response when residing inside macrophages. It is therefore important that *Salmonella* has the ability to efficiently detoxify this potent NO. There are three characterised NO detoxification systems in *Salmonella*, HmpA, NrfA, and NorVW, however deletion of these three genes does not eliminate *Salmonella's* survival under these conditions. It is therefore expected that there are further, yet unknown NO detoxification genes. NsrR, an NO sensing transcriptional repressor, is known to regulate, three putative, tellurite resistance genes in *Salmonella*, *yeaR*, *tehB* and *STM1808*, yet the physiological relevance of tellurite is questionable. We have demonstrated a clear link between the function of these genes in resisting both tellurite and nitric oxide. Of the NO detoxifying systems identified thus far, HmpA is considered to be the most important. We have created a quadruple mutant consisting of the three tellurite resistance genes, *tehB*, *STM1808* and *yeaR*, along with *hmpA*. This strain is acutely sensitive not just to tellurite but also to NO (more so than the single *hmpA* mutant). It is also severely attenuated within macrophages, and we are now developing it as a live attenuated vaccine strain.

S6/P1

Immunoregulatory function of N myristoyltransferase (NMT) during HIV infection

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Protein N-myristoylation refers to the covalent attachment of myristate, a 14 carbon saturated fatty acid, to the N-terminal glycine residue of various mammalian and viral proteins catalyzed by N-myristoyltransferase (NMT). A single gene encodes NMT in lower eukaryotes whereas in humans NMT is encoded by two genes: NMT1 and NMT2. N-myristoylation ensures the proper functioning and intracellular trafficking of proteins. Many proteins involved in a wide variety of signal cascades and cellular transformations are myristoylated. For example, during HIV pathogenesis, NMT localizes HIV virulent factors: gag and nef to the plasma membrane to facilitate virion assembly. Also, during T cell receptor signaling, the early activation signals triggered by TCR engagement are mediated by myristoylated proteins such as LCK and Fyn. Our research findings indicate that NMT1 is the principal enzyme during myelopoiesis. Taken together, we hypothesized that NMT1 may play a role in immune response. To test this hypothesis, we used flow cytometry to analyze NMT1 levels in stimulated $CD3^+$ T cells or unstimulated $CD3^+$ T cells. We observed increased NMT1 levels in the stimulated $CD3^+$ T cells relative to unstimulated $CD3^+$ T cells. Since HIV impairs immune response, expression status of NMT1 was determined in PBMC of HIV infected patients and compared with age and sex matched healthy controls. Our preliminary results indicate down-regulation of NMT1 in PBMC of HIV infected individuals. We report here for the first time alteration in NMT1 expression during T cell activation and down-regulation of NMT1 in PBMC of HIV infected individuals.

S6/P2

Understanding the role of membrane biophysical properties of influenza viruses in virus infectivityLamyaa Al-Dalawi, Cyril Rauch, Stephen P Dunham PhD student,Lecturer, Associate Professor

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Abstract

The influenza virus has a lipid envelope that is acquired from the host cell plasma membrane. The virus envelope plays an essential role during several steps of the virus life cycle, from entry and fusion to replication and virion release (Martín-Acebes et al., 2013). The major aims of this study are to investigate the molecular and biophysical influence of the lipid composition of the virus or host cell membrane on influenza A virus infection. Our research has focused on the infectivity of LPAI H2N3 treated with lipids such as 1, 2-dipalmitoyl-sn-glycero-3-phospho-L-serine (DPPS) and 1, 2-dipalmitoyl-sn-glycero-3-phosphocholin (DPPC). The presence of these lipids had no significant impact on H2N3 infectivity. However, the presence of 1, 2-dipalmitoyl-Sn-glycero-3-phospho-(1'-rac-glycerol) (DPPG) had a significant impact on H2N3 infectivity. Treating the H2N3 influenza virus with various Lyso lipids: 1-palmitoyl-2-hydroxy-sn-glycero-3-phospho-L-serine (LPS), 1-palmitoyl-2-hydroxy-sn-glycero-3-phospho-(1'-rac-glycerol) (LPG) and 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC), all of which contain one aliphatic chain, produced significant inhibition of influenza virus infection of MDCK cells, that was dose-dependent. Exogenous lipids may have an effect in virus morphology that may be the reason of the decrease of H2N3 infectivity pre-treated with lipids. Electron microscopy and HA test were used to investigate virus morphology with or without exogenous lipid treatment; and the binding of the influenza virus to cells in similar conditions, respectively. Our research underlines the lipid composition of the virus as a potential therapeutic treatment for influenza infection

S6/P3

SUMOylation of the Influenza A Virus Matrix Protein 2

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The post-translational conjugation of the Small Ubitiquitin-like Modifier (SUMOylation) regulates a wide array of cellular processes and viruses can take advantage of this for their own benefit to facilitate intracellular trafficking and viral replication. The extent to which this host posttranslational modification is exploited is not fully understood. Several Influenza A viral proteins have reported to be SUMOylated at lysine residues, including non-structural NS1 and NS2, matrix protein 1 (M1), the nucleoprotein NP and polymerase protein PB1. In a pull-down study of 293T cells transfected with A/Udorn/72 viral proteins, followed by western blot analysis, we see that in addition to the known SUMO targets, that matrix protein 2 (M2) and the spliced transcript Variant M4 may also be SUMOylated, possibly affecting protein-protein interactions during virus assembly and budding. Our results suggest that, in addition to the Influenza virus proteins known to be SUMOylated, viral proteins M2 and M4 may also use the post-translational conjugation to exert their functions.

S6/P4

Intracellular metabolism of AIEC contributes to IL-1β production in macrophage

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We have previously shown that glycolysis is important in replication of adherent invasive Escherichia coli (AIEC) in macrophages. AIEC are frequently isolated from patients with Crohn's Disease and are characterized by their ability to invade epithelial cells and replicate in macrophage. Indeed, it has been proposed high levels of cytokine release from AIEC-infected macrophages contributes to the pathology of CD. Production of acetyl-coA from glycolysis is fed into the TCA cycle or converted to acetate. The Pta-AckA pathway converts acetyl-CoA to acetyl-phosphate (AcP) and then to acetate. Here, using a Δpta ackA mutant in AIEC strain HM605, we show that the Pta-AckA pathway is important for i) the intramacrophagic replication of AIEC and ii) the induction of the normal inflammatory response by macrophage infected with AIEC. *pta ackA* mutants accumulate to lower numbers than wild type during macrophage replication and macrophages infected with Δpta ackA release less IL-1β. pta ackA mutants do not make AcP or acetate, therefore we constructed a ΔackA mutant that accumulate AcP. This resulted in lower levels of IL-1β production during macrophage infection suggesting acetate production, and not AcP per se, is required for inducing a full inflammatory response. The Δpta ackA pathway is also found in S. enterica Serovar Typhimurium and infection of macrophages with ΔackA and Δpta ackA mutants resulted in significantly reduced IL-1β release. Therefore, it appears the Pta-AckA pathway or possibly its end product acetate may be a bacterially-derived metabolite that is important for stimulating a normal inflammatory response in macrophages

S8/P1

Isolation and identification of Aflatoxin producing and non-producing strains of Aspergillus

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Aspergillus section Flavi has become focal point of research globally owing to its increasing toxigenicity in food along with wider application in industry. Whereas, identification of species within section Flavi remains challenging due to high level of erraticism in morphological as well as genetic characters therefore, the purpose of this study was to apply polyphasic approach using morphology, extrolite and genetics to characterize Aspergillus isolates in section Flavi. Isolations from different sources lead to morphological characterization of nineteen strains. Identified species were A. flavus, A. flavus var. columnaris, A. oryzae, A. oryzae var. pseudoflavus A. oryzae var. tenuis A. oryzae var. effusus, A. oryzae var. microspores, A. minisclerotigenes and A. parvisclerotigenus. Assessments regarding mycotoxigenic assays through Thin Layer Chromatography (TLC) revealed presence of aflatoxins in thirteen isolates. Amplification of ITS1-5.8S rDNA-ITS2 region produced a PCR product of about 550-650 bp for all strains. BLAST results using ITS sequence of all identified strains showed 100% identity with the many of their respective strains deposited to GenBank. The amplification profiles of ISSR using the P1 (AGAG)4G, P2 (GTG)5, P3 (GACA)4G primers showed an average of 9, 12 and 12 polymorphic bands for the nineteen isolates with band size ranging from 200-1800, 200-1500 and 300-3000bp, respectively. On the basis of amplification banding of ISSR, all isolates were clustered into three clades. A. Present study concludes that proper identification using polyphasic approach is indeed prerequisite that will contribute information in stable taxonomy and nomenclature of A. flavus group.

S8/P2

Mining bacterial phosphonate metabolism to design a biosensor for the herbicide glyphosate

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Glyphosate, the main component of the commercial "Roundup" formulation, is the most widely used herbicide worldwide. It is increasingly evident that glyphosate has detrimental effects on the environment. In-field detection of glyphosate is not currently possible, meaning environmental monitoring is poor and a biosensor is required. Many species of soil-dwelling bacteria are able to break down phosphonates, such as glyphosate, using a C-P lyase enzyme and utilise the products as a source of phosphorous. Uptake of phosphonates requires an ABC transporter which has a periplasmic binding protein called PhnD.We aim to develop a reagentless biosensor to detect glyphosate and its common breakdown product, AMPA, in field and understand more about how bacteria transport and use organic phosphonates. Candidate glyphosate binding proteins have been identified that are PhnD homologues in a diverse range of microbes able to use glyphosate as a sole phosphorous source. Computational approaches were then used to model the predicted structures of these proteins and identify amino acid residues that may be critical to glyphosate binding. Three of these candidate glyphosate binding proteins have been purified from an E. coli expression system. Preliminary isothermal titration calorimetry binding experiments suggest all 3 proteins are able to bind phosphonates. Once proteins have been optimised to bind glyphosate and/or AMPA with high affinity and specificity, this will be used as a scaffold for a reagentless biosensor.

S8/P3

Harnessing the flavour potential of the yeast *Kluyveromyces marxianus*

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Kluyveromyces marxianus is an excellent producer of aromatic alcohols and acetate esters while exhibiting attractive industrial traits such as thermotolerance up to 52°C, high acid tolerance and a very fast growth rate. Coupled with existing GRAS and QPS classification these traits make this yeast very attractive for direct fermentation of novel fermented foods or beverages in either single culture or in combined starter cultures. The potential as a cell factory for over production of flavours and fragrances to make natural additives also is being explored currently. However in order to develop cell factories a more complete understanding of the expression and regulation of these flavour pathways is required. Two novel K. marxianus strains were isolated from repeated cycling of kefir grains in raw milk. Large increases in 'fruit like' aromas were measured. Here we present the influence of different carbon and nitrogen sources on the production of a range of flavour metabolites. The nitrogen source had pronounced effects on metabolite production: levels of the fusel alcohols 2-phenylethanol and isoamyl alcohol were highest when yeast extract was the nitrogen source, and ammonium had a strong repressing effect on production of 2-phenylethyl acetate. In contrast, the nitrogen source did not affect production of isoamyl acetate or ethyl acetate, indicating that more than one alcohol acetyltransferase activity is present. Production of all acetate esters was low when cells were growing on lactose (versus glucose or fructose), with a lower intracellular pool of acetyl CoA being one possible explanation.

S8/P4

Streptomyces formica is a novel antibiotic-producing strain isolated from an under-explored mutualism

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Antibiotic resistance posies a major risk to modern medicine, therefore finding new antimicrobial compounds is vital. Most antibiotics in clinical use today were derived from the natural products of Actinomycetes discovered more than half a century ago. We present a novel Actinomycete, *Strepomyces formica*, isolated from African *Tetraponer*a ants who use the bacteria in a highly evolved mutualism to farm their symbiotic fungus. *S. formica* has antifungal activity against both *Candida albicans* and the emerging multi-drug resistant *Scedosporium prolificans*. Whole genome sequencing and AntiSMASH analysis shows that this talented strain contains up to 38 secondary metabolite clusters. Whilst characterising the antifungal compound, an antibacterial compound was co-purified and shown using LCMS to map to a single Type II Polyketide (T2PK) cluster. Inclusion of sodium butyrate in the growth medium resulted in the production of 13 chlorinated congeners of this T2PK with clear structure-related activity against gram positive *Bacillus subtilis* and *Staphylococcus epidemidis*, but not the gram negative *Pseudomonas putida* or *Escherichia coli.* CRISPR/Cas 9 deletion of the gene cluster confirmed that all the compounds originate from that single biosynthetic pathway. The cluster encodes one halogenase that is predicted to be responsible for chlorination at up to 4 sites on the polyketide chain and appears to mediate antibacterial activity. Further characterisation of this novel actinomycete may produce several compounds of clinical interest. This work demonstrates that searching under-explored environmental niches and combining new genomic techniques to unlock silent natural products is a promising route towards finding new anti-infective agents.

S8/P5

Carbonate biomineral precipitation by *Pseudoalteromonas piscicida* CBRC.15.0062 from the Red Sea

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Carbon dioxide, an abundant molecule in the earth's atmosphere, interacts continuously and dynamically with aqueous systems by speciation into bicarbonate and carbonate depending on environmental factors (e.g. temperature, pH). Calcium ions, common in low concentrations in many environments, have particularly high concentrations in oceans, saline lakes and saline soils. And some industrial effluents have even higher concentrations. Calcium ions can precipitate in a number of mineral forms and more than 60 different carbonate minerals are known.

Many bacterial species from diverse environments have been associated with calcium carbonate precipitation. They are able to influence mineral precipitation and can act as nucleation site and affect the crystal growth. Such capabilities have been receiving considerable interest for its potential applications in different fields (e.g civil engineering, restoration of historical buildings, and remediation of soils).

During the analysis of epiphytic bacteria, collected from biofilms from the leafs of *Halophyla sipulacea* in the Al-Kharrar lagoon (Saudi Arabia) we identified a particularly interesting strain of *Pseudoalteromonas piscicida*. When grown in agarised media, we noticed a high concentration of large biominerals. We isolated these crystals and performed extensive characterisation studies (using e.g. SEM, TEM and XRD).

This is the first report of biomineral precipitation by members of the genus *Pseudoalteromonas*.

S8/P6

Investigating the biology of bacteria capable of inhibiting other microorganisms

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As a result of the ongoing citizen science project "Swab and Send" aimed at identifying new antimicrobial compounds, we have a collection of environmental microbes capable of inhibiting the growth of other microorganisms.

Approximately 2000 environment isolates that were collected from public submissions from around the UK and Egypt, have been screened for inhibitory activity against a range of indicator strains including a MDR clinical Escherichia coli O25b-ST131 isolate, originating from a nosocomial infection in Alexandria, Egypt. Inhibitory isolates to this strain were subsequently screened for activity against MRSA-16 and a clinical C. albicans.

Fifty-two isolates inhibit E. coli O25b-ST131. Amongst these, ten also showed inhibition against MRSA-16, five showed inhibition against C. albicans, whilst two inhibited both MRSA-16 and C. albicans.

Most of these isolates have been identified as belonging to the genus Bacillus, and their phylogenetic relationships have been mapped using a combination of 16S and gyrB typing, MALDI-TOF, and RFLP analysis.

Here we present an overview of our findings using a combination of analytic chemistry, bioinformatics, and next generation sequencing aimed at identifying the antimicrobial compounds produced by these isolates.

S8/P7

Partial Purification and characterization of a chitinase enzyme (10.5kDa) from culture supernatant of *Streptomyces chilikensis* RC1830

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Chitinases are glycosyl hydrolases that catalyze hydrolysis of chitin molecule releasing NAG as the product. In nature these enzymes are produced by bacteria , fungi, actinomycetes and others. The hydrolytic products of chitin have gained special interest in agricultural and food industry. The genus *Streptomyces* (a type genus of the family Streptomycetaceae, is the largest of the phylum Actinobacteria (Kampfer,2006) .

Chilika Lake is the largest coastal estuarine brackish water lagoon in Asia situated on the east coast of India and is a designated Ramsar site.

In the current study, several chitinolytic microorganisms were isolated and screened by appearance of clearance zone on 0.5% colloidal chitin agar plate. A strain designated as RC 1830 displayed maximum colloidal chitin degradation by release of 112 µmol/ml/min of N-acetyl D-glucosamine (GlcNAc) in 48h. The strain was taxonomically identified by polyphasic approach based on a range of phenotypic and genotypic properties and was found to be a novel species named *Streptomyces chilikensis* RC1830. The organism was halophilic (12% NaCl w/v), alkalophilic (pH10) and was capable of hydrolyzing chitin, starch, cellulose, gelatin, casein, tributyrin and tween 80. The partial purification of chitinase enzymes from RC1830 was performed by DEAE Sephacel anion exchange chromatography which revealed the presence of a very low molecular weight chitinase(10.5kD) .The study reports the presence of a low MW chitinase (10.5kD) and a chitin decaetylase from a novel *Streptomyces* strain RC1830 isolated from Chilika Lake.The enzymes was characterized for optimum pH, temperature, and substrate specificity and temperature stability.

S8/P8

New species of *Actinokineospora* and *Streptosporangium* from rhizospheric soil with antimicrobial activity

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The discovery of new antibiotics has been greatly diminished for decades since 1960s, while the emergence of multidrug-resistant microbial pathogens advances. Therefore, finding new therapeutic compounds has still been of scientific interest. One of the well-known characteristics of filamentous bacteria in the actinomycetes group is their ability to produce a wide variety of antibiotics and other classes of biologically active secondary metabolites. Many non-streptomycetes were screened from rhizospheric soil samples and characterized. The taxonomical characteristics of strain 44EHW^T in the genus *Actinokineospora* and strain 30EHS^T in the genus *Streptosporangium* were analyzed. These two strains were proposed as novel species *A. bangkokensis* (type strain 44EHW^T = BCC 53155^T =NBRC 108932^T) and *S. jomthongense* (type strain 30EHS^T = BCC 53154^T = NBRC 110047^T), respectively. The taxonomic studies were based on the morphological characteristic, chemotaxonomic profile and phylogenetic analysis. Antibacterial (*Staphylococcus aureus* ATCC 25923) and antifungal (*Colletotrichum gloeosporioides* DoA d0762) activities were also detected in both strains. This study suggests that novel species of rare actinomycetes might be promising microbial resources for novel metabolites with potential medical applications.

S8/P9

Spirotetronate antibiotics from *Actinomadura* strain 2EPS with inhibitory activity against *Clostridium* species

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Actinomycetes are well known for their capability on producing important secondary metabolites with diverse biological activities such as antimicrobial and immunostimulant activities. The rare actinomycetes strain 2EPS was isolated from Thai rhizospheric soil and was analyzed for their cultural, morphological characteristics, diaminopimelic acid content of its cell wall, and 16S rRNA gene sequence which indicates that it belongs to genus *Actinomadura*. In addition, phylogenetic tree also confirmed the relationships of this strain to other members of *Actinomadura*. A butanol extract with antibacterial activity was purified by reversed-phase chromatography to obtain three bioactive compounds, designated as compounds **1**, **2** and **3**. The structures of these compounds were determined using spectroscopic analysis (1H-NMR and ^{13}C-NMR) and mass spectrometric analysis (HR-TOF-MS). Compounds **1-3** were identified as spirotetronate antibiotics. All compounds were active against Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, and *B. subtilis* ATCC 6633) with low MIC values between 0.08-5.0 µg/ml. Moreover, both 1 and 3 also exhibited strong activity, with similar MIC values, against *Clostridium perfringens* S107 at 0.63 µg/ml and *C. difficile* 630 at 0.08 µg/ml. These results suggest the identified spirotetronate compounds may have potential in the treatment of *Clostridium* infections. Overall, this analysis demonstrates that rare actinomycetes are a promising source for discovery of antimicrobial compounds.

S8/P10

Assessing the impact of intrinsic and extrinsic parameters on ethanol and biomass yields in the yeast *Kluyveromyces marxianus*

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The yeast *Kluyveromyces marxianus* is used in industrial processes for production of biomass, ethanol and other products. Although some traits of the species are well-described – for example rapid growth and thermotolerance, there is insufficient knowledge on the physiological behavior of the species during fermentation. Furthermore, theknowledge available tends to be restricted to single strains whereas it is known that considerable diversity exists within the species. One of theinteresting questions that remains to be resolved is why some strains of *K. marxianus* produce ethanol during batch culture, whereas others do not? The extent to which environmental parameters affect thischoice is also unknown. Phenomena such as the Crabtree effect, glucose repression, overflow metabolism and respiratory limitation may all play a role here. A better understanding ofthis is required to optimise strains for either biomass production of ethanol production – either from hexose (first generation) or pentose (second generation) sugars. We are comparing growth parameters of two well-established strains of *K. marxianus* for which genome sequences are available, CBS 6556 and CBS 397. These strains show differentially physiology, for example in lactose utilization and ethanol production. Experiments are carried out in flask and bioreactor cultures to assess how varying sugar concentration, aeration and other factors affects biomassyield andthe fermentation profile. Other industrially-relevant traits, such as acid sensitivity arealso considered.

S8/P11

Fungal cell wall glycans as a nutrient for bacteria in the human gut

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Bacteroides species isolated from the human gut commonly possess large numbers of carbohydrate active enzymes to allow them to access the complex and variable glycans that pass through the gut. These enzymes are arranged in polysaccharide utilisation loci (PULs) with glycan binding proteins and TonB-dependent transporters, to allow import and degradation of each target glycan. Typically, these targets include dietary plant carbohydrates (e.g. starch, pectin and xylan) as well as host carbohydrates (mucins, glycosaminoglycans), but some *Bacteroides* species are also able to use glycans from other microbes found in the gut. Fungal species in the gut, both transient and resident (*Saccharomyces cerevisiae, Candida albicans*), possess complex glycans in their cell wall, predominantly mannan, glucan and chitin. We showed previously that *B. thetaiotaomicron* is able to degrade mannan from *S. cerevisiae*, and present here data describing how it can utilise the unusual mannan from *C. albicans*, as well as the β1,6-glucan which crosslinks the other glycans of the cell wall. The structure of the GH16 endo-glucanase shows how it is able to accommodate the 'hook-like' structure of the β1,6-glucan, and potentially aid access to the dense α-mannan layer. We also describe an enzyme from the GH130 family which has an unusual β1,2 mannosidase activity, allowing it to target the capping residues of *C. albicans* mannan.

S8/P12

Butyrolactone systems for synthetic biology

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The *Streptomyces* species are abundant sources of secondary metabolites of medical interest. Their production is regulated in a growth-dependent manner by small molecules, known as γ-butyrolactones (GBL), which induce a switch-like transition to antibiotic production upon reaching a threshold concentration.

In *Streptomyces coelicolor*, the butyrolactone system involves ScbA, responsible of the first biosynthetic step in GBL production; and ScbR, a GBL-receptor and DNA-binding protein known to regulate actinorhodin, undecylprodigiosin and coelimycin P1 production. The promoter regions of the divergently encoded *scbA* and *scbR* overlap by 53 bp. It is unclear how this overlap affects the overall regulation of antibiotic production, although computational studies have shown that it could be responsible of the fine-tuned regulation in antibiotic production.

This project aims to understand the role of the ScbA/R system in antibiotic production regulation and evaluate its applicability as a tool for synthetic biology. The project has focused on unravelling the effects of the *scbR-scbA* promoter overlap in antibiotic regulation, by uncoupling the divergent promoters of *scbA* and *scbR*, using CRISPR/Cas9-mediated genome editing. We will then compare the transcription profile of the two genes in its natural configuration and that of the uncoupled state for its effect in antibiotic production. The first evaluation of the system as a synthetic biology tool is performed by introducing the ScbA/R system into the genome of *E. coli*. We will measure the transcription levels of both genes and confirm the translation of the enzymes through western analysis and the ability of the strain to produce butyrolactones.

S8/P13

Expression of type II polyketide synthases in Escherichia coli

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Polyketides represent a chemically diverse class of clinically relevant compounds predominantly derived from Gram positive organisms, and as such have received much interest over the last three decades. This interest stems not only from end compound bioactivity but also from the inherent modularity of polyketide synthases (PKSs) pinpointing these biosynthetic complexes as an attractive target to rationally engineer for the production of new-to-nature bioactive compounds.

Efforts to engineer new compounds using type I and type III polyketide synthases have been both extensive and successful; however efforts to do so with type II PKSs have been significantly hampered mainly through the insolubility of core biosynthetic enzymes when expressed in E. coli.

Here we present the first documented example of soluble type II PKS biosynthetic machinery in E. coli and verify its quaternary structure as a dimer. We also demonstrate the previously unknown ubiquity of type II polyketide clusters in phylogenetically diverse hosts and evaluate solubility of associated core biosynthetic machinery in E. coli.

This work serves as a platform for novel compound discovery through rational engineering of type II polyketides in E. coli using newly developed HTP technologies.

S8/P14

Construction of bacterial microcompartments for the synthesis of monoterpenes

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Terpenes are a family of structurally diverse secondary metabolites synthesized via the mevalonate and MEP pathway in plants and are commercially valuable as food flavours, fragrances, biofuels, and medicine, Expression of terpenes in *E. coli* raises a number of challenges regarding the toxicity of pathway intermediates, low enzyme expression, metabolic flux imbalance and contextual dependency. A strategy to overcome these limitations is the compartmentalization of metabolic reactions. In prokaryotes, the encapsulation of metabolic pathways occurs within bacterial microcompartments (BMCs), for example the propanediol and ethanolamine microcompartments in *Salmonella enterica* and *E. coli*. This cellular strategy insulates the cytosolic machinery from the accumulation of toxic intermediates, while simultaneously facilitating the co-localization of enzymes and pathway metabolites in optimal stoichiometries to promote metabolic channelling. Using a synthetic biology approach, we have developed constructs expressing structural genes (*eutSMNLK*) that assemble to form the ethanolamine microcompartment of E. coli. Furthermore, we are re-engineering the Eut microcompartment by incorporation of homologues for *eutLK* from *S. typhimurium* to generate a hybrid metabolosome. To validate microcompartment formation, we have fused a signal sequence derived from the N-terminal sequences of native encapsulated enzymes to the mCherry and Halotag reporters to visualize microcompartment formation using fluorescent microscopy. Using bioinformatics, we have generated a number of synthetic signal sequences as an initial step to develop a library of peptides used for directing the encapsulation of key enzymatic reactions. Through the use of a synthetic compartmentalisation strategy, we aim to reduce heterologous host toxicity and increase the yields of monoterpenes.

S8/P15

Evolving microbial communities for biofuel production.

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Lignocellulose is the most abundant raw material on Earth and a promising carbon source for biofuel production. Unfortunately, industrial lignocellulose degradation is an expensive and energy intensive process. In nature, lignocellulose is efficiently degraded by microbial communities. Determining how these microbial communities function to degrade lignocellulose is a very active area of research. It is hoped that by understanding these processes and identifying the enzymes involved, the efficiency of industrial lignocellulose degradation could be vastly improved.

This project aims to utilise experimental community evolution to produce bacterial communities able to efficiently degrade lignocellulose. Lignocellulolytic bacteria isolated from compost will be evolved in communities with wheat straw as the sole carbon source. The productivity of these communities will be measured regularly and communities showing the most improved productivity following evolution will be analysed using 'omics approaches to identify the enzymes responsible for improved productivity. These enzymes will then be expressed and characterised to determine potential industrial applications. In addition, the activity of species and the interactions between them will be measured throughout evolution to help understand how species are evolving. This project offers interesting opportunities to not only discover enzymes involved in lignocellulose degradation, but to also gain insight into how both species and functional diversity affects the evolution of a bacterial community and its ability to degrade a complex substrate.

S8/P16

Identification and Heterologous Expression of the Vancoresmycin Biosynthesis Gene Cluster from *Amycolatopsis* isolate DEM30355 and Target Determination

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The heterologous expression of bacterial biosynthetic gene clusters (BGCs) is becoming an increasingly important tool for the investigation and discovery of natural products. To date, there are few examples of heterologous expression outside of the genus *Streptomyces*. Here, we present the cloning and heterologous expression of the antibiotic vancoresmycin from the *Amycolatopsis* isolate DEM30355 by *Streptomyces coelicolor* M1152. This is to our knowledge is the first natural product to be heterologously produced from the genus *Amycolatopsis* and at 140 kb may be the largest BGC heterologously expressed. Vancoresmycin, first isolated in 2002, is a linear tetramic acid antibiotic and exhibits potent antimicrobial activity (0.6 - <0.04 µg/mL) towards Gram-positive bacteria. The mode of action (MoA) of vancoresmycin was previously suggested to be interference with the cytoplasmic membrane, based on the structural similarities with other tetramic acid antibiotics. Here, utilizing a membrane depolarization assay it was possible to exclude this MoA, whilst the use of the *Bacillus* LiaRS reporter assay points towards interference in the lipid II synthesis pathway.

S8/P18

Methods for Inducing Antimicrobial Production from Novel Streptomycetes Isolated From Fire Mountain, China

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Actinomycete bacteria remain a continuing source of novel antibiotics, which are urgently needed to control the spread of drug resistant microbial pathogens. This is due to their genomes rich in biosynthetic gene clusters, giving them the capacity to produce bioactive secondary metabolites. However, many of these clusters are silent under laboratory conditions. Methods to induce the activation of these cryptic gene clusters must be investigated, as they may harbor previously undiscovered antimicrobial compounds. Chemical elicitation using a number of compounds has been further investigated as part of the project, to both increase the yield of known compounds and induce the production of unknown antimicrobial compounds. Promising results have been returned with a number of chemical elicitation conditions, with induction of antimicrobial production from a number of strains not previously observed to produce.

It is hypothesized that antimicrobial compounds are produced in nature as part of the evolutionary arms race, to eliminate neighbouring strains competing for nutrients. Subsequently, co-culturing techniques have been explored to investigate how the proximity of strains isolated from the same environment affects antimicrobial production. During co-culturing, production was observed from an increased number of strains that had not previously shown antimicrobial activity in monoculture.

Streptomyces spp. grow naturally on solid substrates as branched mycelia, and difficulties arise when cultivating in liquid. As laboratory conditions differ enormously to the natural environment that Streptomyces spp. are adapted to, creating a simulated micro-environment has been examined, as well as offering a supportive substrate in liquid media, with extremely promising results.

S8/P19

Synthetic Biology for antibiotic discovery and development

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New antibiotics are urgently needed to replace and supplement those eroded by bacteria resistance. Many of the current antibiotics are natural products or their semi-synthetic derivatives. Actinobacteria, in particular, still present a useful source of novel bioactive molecules. However, there are urgent needs to improve the discovery processes to find novel molecules not already developed into clinically useful antibiotics. Synthetic biology and bioinformatics approaches have vast potential for discovery and development of antibiotics and to overcome the common issues with poor growth and poor yield[1].

Screening of an actinomycete collection by Demuris Ltd has identified many strains with interesting antibiotic properties, one of which, DEM32671, produces a broad range of antibacterial compounds. 16S rRNA analysis revealed DEM32671 to be a novel species belonging to the Micromonospora genus. After sequencing the genome (using PACBio sequencing), bioinformatics analysis using antiSMASH[2], identified 53 potential biosynthetic gene clusters. Eight clusters were prioiritised for investigation and identified from a BAC library. Heterologous expression of the clusters in an optimised host and identification of resulting antimicrobial compounds is being undertaken. Refactoring of the cluster will also be conducted to increase the yield utilising synthetic biology approaches guided by bioinformatics analysis[3,4].

[1] Medema M.H. et al.(2011) Nat Rev Microbiol 9(2):131-137.

[2] Blin K. et al.(2013) Nucl Acids Res 41(W1):W204-W212.

[3] Medema M.H. et al.(2014) PLoS Comput Biol 10(9):e1003822.

[4] Medema M.H. et al (2013) Mol Biol Evol 30(5):1218-1223.

S8/P20

Heterologous expression of silent biosynthetic gene clusters from a novel Micromonospora sp. in Streptomyces coelicolor M1152

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The rapid onset of antimicrobial resistance, especially in clinically relevant bacteria, results in a need for new and novel classes of antibiotics which is largely unmet by today's drug discovery. Actinomycetes are a proven source of biosynthetic gene clusters coding for antimicrobial compounds, with many genomes encoding up to fifty clusters. However, under normal culture conditions not all are expressed and many remain silent. The heterologous expression of silent gene clusters is a promising approach for identifying novel natural products including antibiotics.

The genome of a novel (12bp difference to its closest neighbour), slow-growing Micromonospora sp. which produces a number of

antibiotics was sequenced via PacBio (7.5 MB) and analysed using AntiSMASH (1) resulting identification of a total of 53 putative biosynthetic gene clusters. A high molecular weight genomic library was prepared by BioS&T in the form of a PAC (phage P1-derived artificial chromosomes) library. Eight clusters were prioritised based on the likelihood of coding for antibiotics as well as potential novelty. So far, one of the clusters, coding for Type-3 PKS - NRPS was transferred into Streptomyces coelicolor M1152 and ex-conjugants were cultivated on plates. Bioactivity assays showed activity against Micrococcus luteus. Further work will include isolation and identification of the bioactive compound as well as transfer and heterologous expression of the remaining clusters.

1. Blin, K., Medema, M.H., Kazempour, D., Fischbach, M.A., Breitling, R., Takano, E. and Weber, T. (2013) antiSMASH 2.0--a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res, 41, pp. W204-12.

S8/P22

Screening probiotic lactobacilli for pancreatic lipase inhibitory activity for their anti-obesity potential

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Obesity, a worldwide health problem is a result of disequilibrium between energy intake and expenditure and the key strategy to combat it is to prevent chronic positive impairments in energy equation. Since, pancreatic lipase hydrolyses triglycerides to glycerol and fatty acids that leads to obesity; therefore, its inhibition is a potential therapeutic agents for diet-induced obesity. For this, lactobacilli for pancreatic lipase inhibitory activity were isolated from different sources. Samples were enriched in MRS broth for 24h at 37oC. Gram positive and catalase negative rods were selected for genomic DNA extraction and cultures were confirmed as lactobacilli using genus specific PCR. Preliminary identification was done for non-lipase producers, selected isolates were subjected to the inhibitory activity. Out of 52 isolates, 30 were negative for lipase production and 19 were showing pancreatic lipase inhibition. Amongst these 10 cultures were showing good probiotic attributes in vitro and were selected for optimizing the inhibitory activity for time, temperature and inoculum. Five cultures showing maximum activity were confirmed using species specific PCR. It is suggested that lactobacilli having inhibitory potential for lipase along with probiotic characters can possibly be used in controlling obesity. However, *in vivo* studies are under way for more confirmation for better understanding. Moreover, long-term study is needed for the source of bioactivity which can be directly used in animal models or human volunteers to validate the hypothesis.

S8/P23

Metabolomics for system and synthetic biology of actinomycetes

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Actinobacteria are prolific producers of secondary metabolites many of which have significant clinical and commercial value. Identifying these novel compounds is of critical importance and numerous strategies have been employed to identify secondary metabolites from cryptic or newly discovered gene clusters.

Most of these gene clusters are frequently not expressed or are in strains that are difficult to grow. Recent advances in the field of synthetic biology have given rise to the development of plug-and-play strategies, whereby these gene clusters can be expressed in optimised chassis.

Consequently, the development of metabolomics platforms to reliably identify and quantify metabolites is necessary to discover or improve the production of these natural compounds. This project aims to develop a mass spectrometry based metabolomics strategy (to identify untargeted metabolites) to use as a debugging tool for natural and engineered chemical producing strains e.g. *Streptomyces coelicolor*.

Existing extraction strategies for metabolomics sample preparation in *S. coelicolor* will be assessed and optimised, as in most cases a limited number of metabolites are identified using the existing methods. The state-of-the-art LC orbitrap and GC Q-ToF MS will be used to analyse extracts and determine a metabolic baseline of *S. coelicolor*. In parallel, computational tools will be developed to analyse the quantitative data generated by the developed metabolomics analysis.

S10/P1

Detection of viable mycobacteria within 6 h using novel bacteriophage technology

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Detection of mycobacterial pathogens is often hindered by their slow-growing nature, making them difficult to culture; therefore current diagnostic tests for animal diseases such as bovine TB and Johne's disease rely on immunological based-methods that often lack sensitivity. We have developed the bacteriophage amplification assay as an alternative rapid method and this has been used to successfully detect and identify a range of different pathogenic mycobacteria including *M. tuberculosis, M. bovis, M. paratuberculosis* (MAP) in clinical samples within 48 h. The advantage of phage-based detection is that only viable cells are detected, phage are very efficient at extracting genomic DNA from low numbers of cells, allowing sensitive and specific amplification of signature DNA sequences. Here we report, for the first time, a new assay format that simplifies and shortens the detection and identification within 6 h and removes the need for any growth of cultures or the use of agar plates. Using this method we have able to detect MAP and *M. bovis* in the peripheral blood naturally and experimentally infected cattle. In a sample of 41 TB-reactor animals, 95% were positive for MTB Complex bacteria. In contrast no positive results were gained when a set of blood samples from 45 TB-negative cattle were tested. These results indicated that bacteraemia is much more common than currently believed during *M. bovis* infection and that this simple rapid test has the potential to improve detection of these pathogens in blood samples, facilitating the control and further understanding several mycobacterial diseases.

S10/P3

The Effects of Macrophage Polarization and Granuloma Microenvironment on the Macrophage Response to *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis remains a leading health issue, killing 1.4 million people each year. Control programs are hindered by ineffective vaccination, drug treatments that require administration over long periods and the rise of multi-drug resistant strains of the bacterium. Rational development of new therapies will require a better understanding of host-pathogen interaction in TB infection.

Central to the pathogenesis of *M. tuberculosis* is an ability to replicate in host cells, in particular the macrophage. There are many different types of macrophages because the cytokine and biochemical environment affects their differentiation and maturation. However, much of our knowledge about *M. tuberculosis*/macrophage interaction is based solely on studies in M1-like macrophages. Less is known about the interaction with M2-like macrophages and even less about the mycobacterial interaction with macrophages exposed to the high level of lipids and lipoproteins found in the in vivo tubercle lesion.

In this study we compared various features of human macrophages differentiated in GM-CSF or M-CSF and investigated their interaction with mycobacteria. We also studied mycobacterium-host cell interactions in so called Mox macrophages, which were matured in the presence of GM-CSF or M-CSF and the oxidized phospholipid, OxPAPC, which is abundant in the tubercle. Finally, we characterised aspects of mycobacterium infection of foamy macrophages, generated by culture with oleic acid or vLDL, focussing on how this affected bacterial and host cell death.

S10/P4

Programmed necrosis of the *Mycobacterium tuberculosis* infected macrophage

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An important mechanism of *Mycobacterium tuberculosis* pathogenesis is the ability to control cell death in infected macrophages, including inhibition of apoptosis. An alternative form of programmed cell death, necroptosis, has been described although its occurrence in different cell types varies. We demonstrate that *M. tuberculosis* induces RIPK1-dependent necroptosis in human monocyte-derived macrophages and murine J774A.1 macrophages cultured in the presence of excess TNF. Using sh-RNA knockdown of RIP3K, we demonstrated the involvement of RIP3K in *M. tuberculosis*-induced cell death in the presence and absence of excess TNF. Whereas virulent *M. tuberculosis* inhibits chemically induced apoptosis of J774A.1 macrophages, *M. tuberculosis* augments the induction of necroptotic death in macrophages stimulated to undergo necrosis with TNF/zVAD. Necroptotic death in macrophages displayed characteristics of necrosis, macrophage activation, and autolysosome formation, but was not associated with enhanced phagosome-lysosome fusion or an increase in antimycobacterial capacity of the macrophage. This is consistent with a scenario whereby virulent *M. tuberculosis* actively simulates necroptotic cell death in the presence of excess TNF to enhance escape to the permissive extracellular milieu and maximise tissue pathology to aid the infectious cycle.

S10/P5

Modelling drug-tolerant mycobacterial sub-populations using a low shear, micro gravity culture system

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Tuberculosis (TB) is a bacterial disease that primarily infects the lungs which is caused by the bacillus *Mycobacterium tuberculosis (M.tb)*. Approximately 1 in 3 people are infected globally with 1.5 million people dying from TB in 2014 alone. Extended treatment times (>6 months) with at least four antimicrobial drugs are required so as to remove *M.tb* sub-populations that persist throughout drug therapy. Despite the hypothesised clinical significance of drug tolerant *M.tb* sub-populations in vivo current drug development models for TB fail to account for these complex mycobacterial populations.This project aims to develop an *in vitro* culture system that represents the *M.tb* sub-populations present in the human lung at which chemotherapy is targeted.

This work utilises a three-dimensional Rotary Cell Culture System (RCCS) to model mycobacterial growth in low shear conditions. *Mycobacterium bovis* (*M. bovis*) BCG was cultured in the RCCS to induce biofilm formation, alongside non-rotating control cultures that did not form biofilms. Planktonic *M. bovis* BCG derived from the RCCS-biofilm model exhibited isoniazid and streptomycin but not rifampicin drug tolerance compared to control planktonic cells. Subsequent passaging of drug-tolerant bacilli derived from the RCCS in antimicrobial-free media showed that the observed drug tolerance of RCCS derived biofilm cells is phenotypic than genotypic.

This low shear *in vitro* model may more accurately represent *in vivo* drug-tolerant *M.tb* populations than traditional *in vitro* models. Further understanding of the physiological state of mycobacteria in models that mimic the *in vivo* environments of the human lung will contribute to novel drug development strategies.

S10/P6

Mycolic acid processing in *Mycobacterium tuberculosis* Asma Javid, Rainer Kalscheuer and Apoorva BhattSchool Of Biosciences, University of Birmingham

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Mycobacterium tuberculosis the causative agent of tuberculosis (TB) infects upto 9 million people per year resulting in approximately 1.5 million deaths due to the disease. With the emergence of multi drug resistant (MDR) and extremely drug resistant (XDR) strains arises the need for novel targets for anti-TB therapy. Mycolic acids are essential components of the unique, lipid rich cell wall of *M. tuberculosis.* However, enzymes involved in the biosynthesis of mycolic acids remain under exploited as drug targets despite one of the early and hallmark anti-TB drug isoniazid which inhibits mycolate biosynthesis. Previous studies from our laboratory identified mycolate processing enzymes and transporters. Using gene knockdowns we have now extended these studies to slow growing mycobacteria like Mycobacterium tuberculosis. Furthermore using BLAST-P alignments and predictions of a 3D structure we identified unique domains in the mycolate processing enzymes, and present functional studies on the same.

S10/P7

Identification of Novel Bovine Tuberculosis Control Approaches Using a Transposon Mutagenesis Approach

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Bovine tuberculosis (bTB) is a huge animal health concern in the UK and the development of a cattle vaccine is a research priority. Although modelling studies suggest that vaccination of cattle may be an important part of an integrated bTB control programme, vaccination of cattle with BCG offers only 60-70% protection. In this study, we use a transposon screening approach to identify antigens that could be added to the current BCG vaccine, as a boosting subunit vaccine, to improve protective efficacy. We constructed a saturated transposon library of Mycobacterium bovis BCG and assessed survival of mutants in BCG vaccinated and immunologically naïve cattle using an intranodal challenge of cattle model previously developed. 3 weeks post challenge, lymph nodes were harvested and the surviving transpsoson mutants were identified by next generation sequencing. Following this, tranpsoson mutants exhibiting increased survival in vaccinated animals compared to naïve animals will be identified. We hypothesise that some of the surviving mutants will lack important protective antigens. The genes corresponding to these protective antigens will then be cloned and over expressed in E. coli. In order to evaluate the potential of these antigens as subunit vaccines, the immunogenicity of the proteins will be assessed using blood samples from BCG vaccinated or M. bovis infected cattle.

S10/P8

Mycolactone, the lipid virulence factor of *Mycobacterium ulcerans* causes an atypical stress response which underlies its cytotoxic effects.

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Buruli ulcer (BU) is a progressive necrotising skin disease caused by *Mycobacterium ulcerans*. The bacteria produce a lipid virulence factor, mycolactone which is responsible for the cytotoxicity and inhibition of the immune system seen in the disease.

The precise molecular mechanism involved in mycolactone mediated cell death is not known. However we recently showed that it blocks the translocation of proteins into the endoplasmic reticulum (ER) via the Sec-61 translocon. Translational profiling carried out on mycolactone treated cells revealed that it causes an increase in the translation of ATF4, CHOP and GADD34 which are involved in the integrated stress response (ISR).

We investigated the effect of mycolactone on the pro-apoptotic eIF2α/ATF4 pathway and have determined that it induces an ISR by activating this pathway. Furthermore treatment of ATF4 knockout cells with mycolactone led to a significant decrease in the rate of cell death. Despite the fact that mycolactone interferes with the biochemistry of the endoplasmic reticulum (ER), it does not cause phosphorylation of PERK, increased expression of BIP, or XBP1 splicing. All of these are hallmarks of the ER stress response, therefore suggesting that mycolactone does not cause ER stress. Thus our results suggest that mycolactone induced ISR contributes to its cytotoxic effect.

S10/P9

Differential Modulation of Cytokine Expression by ERK and p38 MAP Kinases in response to *Mycobacterium avium* Infection in Human and Chicken Cells

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There are limited data on the innate response to *M. avium* infection in poultry. Several cell signaling pathways are involved in the mediation of expression of a number of cytokines induced by mycobacterial infection in mammals. The mitogen activated protein kinases (MAPK) pathway play a crucial role in this process and they are important in the pathogenesis of mycobacterial infection. In this study we evaluated the roles of ERK and p38 MAPK pathways in innate responses to *Mycobacterium avium* in both human and avian macrophage-like cells.

Cells were infected with eight clinical isolates of *M. avium*. The cells were pre-treated with highly specific inhibiters of the ERK (PD98059 or U0126) and p38 (SB203580) prior to infection and the levels of cytokine production at 6 and 24 h post infection were assessed. Pro-inflammatory cytokine production in response to infection was measured in THP-1 cells by ELISA and in HD11 cells by RT-qPCR.

M. avium infection resulted in differential expression of cytokines and chemokines between isolates. Treatment of the cells with ERK pathway inhibitors inhibited induction of cytokines in both human and avian cells. Inhibition of p38 pathway inhibitors modulated cytokine production to different extents. The results suggest some significant differences between signalling events between avian and human cells following *M. avium* infection.

S10/P10

Epidemiological characteristics and clinical outcome of HIV-related tuberculosis in a population of TB patients in Southwestern Nigeria

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Background: Tuberculosis constitutes a major cause of illness globally and is the second cause of death from infectious disease. The impact of this infection is more dramatic in resource limited settings such as sub-Saharan Africa. Individuals who are infected with the Human immunodeficiency virus who also develop tuberculosis represent a significant challenge to the control of TB.This study was carried out to determine the prevalence of TB/HIV coinfection and pattern of infection among patients being managed for tuberculosis. We also compared treatment outcome among co-infected patients with those not coinfected.

Methods: A six year retrospective review of records of patients managed at the Tuberculosis treatment center of the LAUTECH Teaching Hospital, Osogbo, in South-Western Nigeria was carried out. Demographic and clinical information were extracted for analysis.

Results: One hundred and five (26.3%) of the three hundred and ninety nine patients treated for TB in the period of study were coinfected with HIV. About ten percent (9.5%) of the subjects had extrapulmonary tuberculosis which not significantly different from that in co-infected individuals (5.7%). Treatment failure was significantly worse among patients who had both HIV and TB compared with those who had TB only (49.5% vs 32%, p=0.001). Death rate was also higher in the HIV coinfected individuals.

Conclusion: TB patients who are coinfected with HIV experience a poorer clinical outcome and the prevalence is high in this environment. Control of HIV/AIDS will result in improvement of in the morbidity and mortality associated with tuberculosis.

S10/P11

Biosynthesis of mycobacterial lipoarabinomannan: disruption of aftB results in complete loss of terminal β(1→2) arabinofuranosyl residues

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Mycobacterium tuberculosis, the etiological agent of tuberculosis, remains a highly successful bacterial pathogen. Its persistence is associated with the thick, carbohydrate and lipid rich cell wall with distinct lipoglycans that enables *M. tuberculosis* to survive under hostile conditions, such as shortage of nutrients and antimicrobial exposure. The key features of this highly complex cell wall are the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex and phospatidyl-*myo*-inositol derived lipoglycans with potent immunomodulatory properties, notably lipomannan and lipoarabinomannan (LAM). AG and LAM (lipo)polysaccharides share similar arabinan domains with a well-defined structure, however, the biosynthesis of both remains somewhat incomplete. Mycobacterial glycosyltransferases with dual functionalities that are involved in LAM biosynthesis in addition to AG have been successfully identified. Here, we have investigated the potential role of known arabinosyltransferase AftB in the biosynthesis of LAM. Deletion of *aftB* in *Mycobacterium smegmatis* could only be achieved in the presence of a rescue plasmid carrying a functional copy of a gene, strongly suggesting that *aftB* is essential. Lipid analysis of the conditional mutant strain demonstrated an increase in cell wall associated lipids and a significant decrease in cell-wall bound mycolic acid methyl esters. A monoclonal antibody generated against hexa-arabinan motif of LAM no longer recognized LAM extracted from the conditional mutant strain. Subsequent structural characterization of this LAM demonstrated a lack of β(1→2) arabinofuranosyl residues. All together, our results shed further light on the complexities of *Mycobacterium* cell wall biosynthesis, such as in *M. tuberculosis* and related species and represent a potential new drug target.

S10/P12

Investigating a new mechanism of action for a chloramphenicol derivative in Mycobacteria

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Mycobacterium tuberculosis is the causal organism of tuberculosis (TB), a bacterial infection mainly affecting the pulmonary system of the human body. In 2013, the WHO reported that 1.5 million people died around the globe due to TB infection, including people co-infected with HIV. The WHO endorsed the Direct Observed Treatment Shortcourse (DOTS) program, to control morbidity and mortality due to TB. However, the emergence of drug resistant strains against first-line and second-line drugs have developed; leading to MDR, XDR and TDR-TB. It is vitally important that we rapidly discover new drugs and new drug targets. In this regard, we screened a library of FDA approved drugs against both fast growing and slow growing species of *Mycobacteria* to determine whether the current pharmacy of drugs might harbour molecules with anti-mycobacterial activity to the same level of potency as the current TB drug arsenal. We identified florfenicol as one of a number of hits that was seen to be affective against both *M. smegmatis* and *M. bovis* (BCG). Interestingly, the apparent mode of action of florfenicol appears to target a putative enoyl-CoA hydratase EchA12, rather than the more obvious targeting of the ribosome, much like chloramphenicol. Further investigation into the mode of action of florfenicol together with the biochemical characterization of EchA12 will provide further clarity on the role of this protein and determine whether florfenicol has the potential for further drug design and development.

S10/P13

Rapid phenotypic susceptibility testing of bacteria: SLIC by name and slick by nature

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Tools to detect the susceptibility of bacteria rapidly are necessary if we are to turn the tide of increasing antimicrobial resistance. Until a comprehensive set of molecular diagnostic tools are produced phenotypic resistance will remain necessary.

We have integrated several technologies in our novel susceptibility methodology. SLIC: Scattered Light Integrated Collector is a method of detecting very small number of bacteria (10-100 per millilitre) which we harness to determine susceptibility. We present data that shows that we can distinguish phenotypic susceptibility from resistance in approximately two generations. This means that a result could

be available in a much shorter time than conventional methodologies: E. coli- 45 minutes, S. aureus- 60 minutes, K. pnuemoniae- 60 minutes, S. pyogenes- 45 minutes, B. subtilis- 75 minutes. The testing platform is robust and could be automated it is, moreover, easy to produce at high volume and low cost.

SLIC susceptibility compares favourably with the published turnaround times of devices such as the Vitek 2 (TTP in >500 minutes), the Phoenix (TTP in >600 minutes) and the Microscan walkaway (TTP in >1100 minutes).

Detecting susceptibility rapidly is essential. A tool such as SLIC-susceptibility could be the ideal partner for MALDI-TOF identification allowing a comprehensive organism characterisation within an hour of isolation. Such a development could transform the diagnostic landscape

S10/P14

What is the role of the PEP-pyruvate oxaloacetate node of metabolism in the intracellular life cycle of M*ycobacterium tuberculosis*?

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Metabolic reprogramming in response to the host niche during both the acute and chronic phase of tuberculosis infections is a crucial determinant of virulence. Experimental evidence has identified central carbon metabolism as instrumental in this pathogenic strategy. In *Mycobacterium tuberculosis* the enzymes, PEP carboxykinase (PCK), pyruvate carboxylase (PCA), malic enzyme (MEZ) and pyruvate phosphate dikinase (PPDK) act as an anaplerotic node inter-connecting the main pathways of central metabolism (glycolysis, gluconeogenesis and the TCA cycle). This node is critical to the distribution of carbon flux within central carbon metabolism. Using a combination of mutants and ^{13}C isotopomer analysis we demonstrate the importance of this node to the survival of *M. tuberculosis* in macrophages. Although PCK is able to compensate for the loss of PCA in the node, PCA is synthetically lethal when deleted from a PCK knock out strain. ^{13}C isotopomer analysis revealed that this is due to the essential role of PCA in CO_2 fixation. We further show that *M. tuberculosis* requires both a functional PPDK and a functional MEZ for intracellular survival. Our work provides further evidence for *M. tuberculosis'* requirement for both gluconeogenesis and CO2 fixation during intracellular survival. The data presented shows that a fully functional anaplerotic node is required for *M. tuberculosis* to survive and replicate intracellularly. Targeting the anaplerotic node is an interesting therapeutic approach against this pathogen.

S10/P15

Strain variation in the qRT-PCR transcript signals from *M. tuberculosis* growth in biofilms; strategies for standardisation and normalisation.

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Tuberculosis remains a major global health problem. Our group has been studying the phenotypes of *M. tuberculosis* (Mtb) in sputum from TB cases to gain insight into the bacterial populations potentially involved in transmission and those we need to eliminate by treatment. In trying to understand the environmental conditions responsible for the bacterial transcriptional and cytological profiles we have reported in sputum we have explored the influences of different growth conditions including biofilms.

Initial work with MtbH37Rv incompletely replicated the features seen in sputum. Because this strain has not had to survive transmission for nearly a century, we hypothesised that some of the shortcomings of our results might reflect strain differences. Thus we sought to compare the pattern of abundance of selected transcripts and mycobacterial lipid bodies in biofilm growth of three relatively recent clinical isolates of Mtb (Beijing, South Asian and *M. africanum*) with those features observed in H37Rv.

We encountered significant difficulties in obtaining comparable growth patterns and in choosing appropriate qRT-PCR normalisation strategies. We have compared use of multi-housekeeping genes (e.g.Normfinder), *16S* , *SigA* to normalise and have settled on the last of these. We confirm substantial inter-strain differences in the phenotypes observed and discuss the implications of our results.

S10/P16

With the greatest of ease? – The tubercle bacillus and its transcriptome in flight.

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Tuberculosis (TB) kills 1.1 million people each year and is

transmitted by patient-generated aerosols, but little is understood about its adaptations to the airborne state after residence in the host lung.

We used the cough aerosol sampling system (CASS) to study *M. tuberculosis* aerosolised by patients in Pretoria. AFB smear and GeneXpert-MTBRIF positive patients were sampled during two five minute bouts of voluntary coughing; contemporaneous sputum samples were also taken. RNA analysis was by qRT-PCR, RNA-seq and microarray.

Four samples from three patients yielded sufficient RNA for sequencing; only one sputum proved suitable for gene expression profiling. Rockhopper analysis indicated >100 transcripts were differentially expressed ($q<0.01$) between aerosol and sputum. Key findings include evidence of DosR activation, the up-regulation of multiple Toxin-Antitoxin systems and reduced levels of expression of ribosomal protein-encoding genes in aerosol. Given the short period between expectoration and sampling (probably <30 seconds), we suggest that the difference reflects the selection of a sub-population of the tubercle bacilli that have recently been aerosolised in the airways, rather than the direct sampling of sputum. Analysis of the phylogenetec origins mycobacterial and non-mycoobacterial 16S transcripts in four matched aerosol and sputum samples further support this view. We conclude that aerosol is distinct from sputum; our approach opens up new opportunities to elucidate the biological basis for the transmission of TB.

S10/P17

Interrogating cytochrome P450 structure, function and druggability in Mycobacterium tuberculosis

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The human pathogen Mycobacterium tuberculosis (Mtb) causes more human deaths than any other infectious disease. The prevalence of drug- and multidrug-resistant strains of Mtb has compounded the problem and has led to recent research to produce new classes of antibiotics. Mtb has an unusually large number of cytochrome P450 (P450) enzymes, with functions including cholesterol metabolism, novel secondary metabolite production and menaquinone hydroxylation. P450s essential for bacterial survival in the macrophage are particularly attractive as novel Mtb drug targets. As a novel approach to developing Mth therapeutics we have undertaken fragment based screening studies against various Mtb P450 isoforms, producing compounds with high affinity for the targeted enzymes as effective new inhibitors. Recent studies on the Mtb P450s have revealed that selected isoforms are produced from leaderless mRNAs to generate variant P450 enzymes of shorter length than the annotated database sequences. Expression of these "truncated" P450 enzymes (for example, in the case of the Mtb CYP144 isoform) produces robust, heme-bound P450 proteins, suggesting that Mtb has the capacity to express different forms of various P450s, and perhaps explaining the unusually large predicted size of several of the Mtb P450s in comparison with various other bacterial P450s. Progress in the analysis of the structural and catalytic properties of the Mtb P450 enzymes will be presented, along with novel data on inhibition strategies.

S10/P18

Identification of genes required for *Mycobacterium bovis* BCG persistence in cattle.

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The only vaccine with proven efficacy against tuberculosis in either humans or animals is *M. bovis* BCG. Integral to its use in animals, is the development of a diagnostic skin test that can be used in combination with the vaccine. Current skin tests are incompatible with the BCG, as vaccination elicits a response that does not distinguish between infection and vaccination. Other antigen-based tests, which utilise the antigens lost from virulent *M. bovis*, during its transition to avirulent BCG, remain expensive and inappropriate for agricultural situations. This work aims to tackle this problem by developing both an antigen-depleted BCG vaccine, to be used in combination with a new defined skin test.

The first step in the development of this system is to identify antigenic genes that are not required for BCG to persist in the host. By inoculating cattle with a BCG transposon library we identified genes whose removal impacts upon BCG's ability to persist in cattle. Amongst these genes are many known to be involved in mycobacterial virulence, including genes involved in pDIM and sulpholipid synthesis, genes of the mce1 and mce4 regions, and those involved in nitrate reduction and metal transport. Importantly, very few genes encoding putative diagnostic antigens were important for persistence in the cattle. These insights demonstrates that the development of a novel skin test using defined antigens in combination with a modified BCG is feasible, without restricting the ability of BCG to persist in host.

S10/P19

Modelling mycobacterial lipid bodies in *Corynebacterium glutamicum*

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Mycobacteria are characterised by high lipid content, located in the cell envelope and intra-cytoplasmic lipid bodies (LBs). The exact physiological roles of mycobacterial LBs are yet to be defined. Like eukaryotic lipid droplets, their major component is the neutral lipid triacylglycerol (TAG). LBs perform a role in carbon storage, but may also have a more dynamic role in maintaining the flux of long-chain fatty acids to support growth. TAG-LBs are central to the metabolism of *Mycobacterium tuberculosis*, which causes tuberculosis, a disease claiming over one million lives annually. TAG-LBs, a prominent feature of tubercle bacilli in patient sputum, show promise as biomarkers for antibiotic tolerant persister populations and as an adaptation to transmission. Understanding the biochemical and cytological processes involved in LB manufacture, assembly and mobilisation would provide new opportunities for the control of mycobacterial infection.

Mycobacteria have high redundancy in LB-related enzymes, making our knowledge of their individual contributions to LB biology uncertain. Furthermore, direct identification of LB-associated proteins in isolated LBs is likely to be complicated by other unrelated 'bystander' proteins. We therefore sought to develop a biological system in which LBs can be studied on a neutral lipid and LB-free background. We have established such a synthetic biological model in *Corynebacterium glutamicum.* Corynebacteria cannot synthesise TAG, having no homologues of triacylglycerol synthase (TGS) enzymes and are LB-null. Through heterologous expression of the *M. tuberculosis* TGS, Tgs1, we have established TAG synthesis and LB formation in *C. glutamicum* and report here the characterization of this model system.

S10/P20

Understanding the regulation of host mRNA translation during microbial infection.

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Tuberculosis (TB) is a major health problem worldwide resulting in 1.4 million deaths. The control of tuberculosis has been severely thwarted by the emergence of multidrug and extensively resistant strains of the causative agent, *Mycobacterium tuberculosis* and novel strategies are required for treating and preventing this disease. Host-directed TB therapies are an attractive route that could enhance disease resolution, improve treatment outcomes and reduce duration of therapy. The development of host directed therapies will be expedited by further understanding of how this pathogen hijacks host cell processes to facilitate survival. Key to this process is the regulation of host gene expression. However, very little is known about translational control by bacterial pathogens, including *M. tuberculosis* and how this contributes to pathogenesis.

Our aim is to dissect how *Mycobacterium* alters translation in the infected cells, and how the regulation of eIF4E activity participates in this response to infection. Our results suggest that during mycobacterial infection of macrophages, the MAPK pathway activates eIF4E phosphorylation. Furthermore, the kinases ERK and Mnk are responsible for eIF4E phosphorylation and their activation contributes to changes in the translational state of host mRNAs, as identified by polysome profiling. These changes alter the macrophage response to mycobacteria, affecting intracellular bacterial survival and macrophage viability. We propose that regulating eIF4E phosphorylation is a key component of the host-pathogen interaction during Mycobacterium infection.

S10/P21

Mechanism of transcription initiation in actinobacteria

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Transcription initiation is catalysed by a multi-subunit RNA polymerase, which is also the cellular target of rifampicin and related antibiotics. Our understanding of this process derives primarily from research on the *Escherichia coli* enzyme and has shown that promoter interactions and initiation is directed by the sigma subunit. However, recent studies have shown that *Mycobacterium tuberculosis* and other actinobacteria (e.g. *Streptomyces*) differ from this paradigm in requiring two additional essential transcription factors – CarD and RbpA. Each stimulates transcription by contacting DNA upstream of the -10 promoter element and stabilising transcription initiation intermediates. New structural and biochemical insights will be presented that explain how each of these two factors associate with RNAP and globally impact on gene expression.

S11/P1

Experimental evolution of a conjugative plasmid in diverse *Pseudomonas* species

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Conjugative plasmids are able to transfer advantageous traits such as antibiotic and heavy metal resistance between phylogenetically diverse hosts and drive bacterial adaptation and diversification. However, it is still enigmatic how conjugative plasmids are sustained in a community given that (1) plasmid maintenance is costly; (2) plasmids can be lost by segregation; (3) even if plasmids do carry beneficial genes, those genes can be integrated into the chromosome, so the plasmid-borne genes are no longer beneficial for the plasmid's selection. In order to resolve this paradox, we use the conjugative pQBR plasmids that carry mercury resistance genes in the Tn5042 transposon and various Pseudomonas species as plasmid hosts in an experimental system. In this study, we experimentally evolved the ~425 kb conjugative plasmid pQBR103 using 5 different *Pseudomonas* species (*P. fluorescens* SBW25, *P. putida* KT2440, *P. aeruginosa* PAO1, *P. syringae* pv. phaseolicola, *P. stutzeri* JM300) as plasmid hosts. Replicate populations were experimentally evolved for 120 days in the presence and absence of mercury which allowed us to track the changes in the plasmid's life-history in the different plasmid-hosts. *Pseudomonas* species varied greatly both in their ability to stably maintain the plasmid and in their propensity to integrate the mercury resistance transposon onto the chromosome, which occurred both with and without mercury selection. These results suggest that in natural communities only a subset of potential host species are likely to be responsible for the long-term survival of conjugative plasmids.

S11/P2

Understanding cross-resistance and its implications to phage therapy

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The ability of phage to counter-adapt against bacterial resistance has made them an appealing prospect in the development of phage therapy as an antibiotic treatment. Many phage therapies are cocktails of multiple phage strains aimed to target a broad range of bacterial targets. Antagonistic co-evolution between bacteria and phage has been studied for many years in a pairwise context, but we know less about the nature of co-evolution in a community context and how resistance would develop against a diverse group of phage. Using a collection of Pseudomonas aeruginosa strains and associated lytic phage strains isolated from cystic fibrosis patients we are exploring the occurrence of cross-resistance, where the evolution of resistance to one phage strain prevents infection by other phage strains. We have found surprisingly high levels of cross-resistance implying that the design of phage cocktails needs to be considered carefully. Characterisation of the bacteria and phage strains enables us to investigate a range of associations, such as the connection between generalist/specialist phage host ranges and the resistance mechanisms they promote. Using bacteria-phage infection networks we can design phage cocktails based on grouping generalist or specialist strategies and varying overlap of host ranges to explore the effectiveness of different design strategies.

S11/P3

Methylation warfare: interaction of streptococcal bacteriophages with their host

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Phase variation is an important mechanism contributing to the virulence of numerous human pathogens including *Streptococcus pneumoniae*.The pneumococcal Type I restriction modification system SpnD39III acts as a defence mechanism against infection by the novel temperate bacteriophage SpSL1. We have previously demonstrated that SpnD39III is phase variable and switches between six alleles with different DNA binding specificities and virulence phenotypes. Different alleles are therefore able to restrict infection by bacteriophage to those viruses displaying the host's methylation pattern. Cell death and cell lysis still occurred in these infections however, suggesting there is activation of an abortive infection (Abi) response. Surprisingly, the Abi response is also dependent on a functional SpnD39III system. Expression profiling of SpSL1 by RNAseq revealed increased expression of the C5 cytosine methyltransferase gene during abortive infection, indicating that phage-mediated methylation may also play a role in cell death. We sought to further investigate the host cell lysins and phage- and host-encoded methyltransferases to reveal the relative contributions each makes to abortive infection. This will contribute to our understanding of the complex dynamics of the bacterial-virus interaction.

S13/P1

Stabilising the empty capsids of poliovirus by thermal evolution

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Poliovirus is the causative agent of poliomyelitis, a highly infectious viral disease, which can result in paralysis and may lead to death. There are two current vaccines against the disease i.e. oral polio vaccine (OPV) and inactivated polio vaccine (IPV). Owing to OPV-associated polio outbreaks and the large scale culture of live virus required to produce IPV, both vaccines have the potential to become biological hazards post eradication. There is a drive towards development of a safer vaccine which will be ideal for a polio-free world. Empty capsids are produced as part of the viral lifecycle but are antigenically unstable. If stabilised, these could be an ideal future candidate virus-like particle vaccines. In this study, we have selected heat-stable mutant polioviruses, resulting in viruses with empty capsids that have similar stability to current polio vaccines. We have shown that two key mutations in structural protein VP1 (I1194V and V1087A) evolved over cycles of thermal selection and were maintained within all populations of selected viruses. Mutations in other structural proteins (VP3 and VP4) were also identified e.g. a transient VP4 (R4018K) mutation which was identified in the population when under increased selection pressure. Work is in progress to understand the pattern of evolution of the mutations and also to produce these stabilised virus-like particles in recombinant expression systems as candidate virus-free vaccines.

S13/P2

Characterisation of the infectious bronchitis virus replication transcription complexSelma Rayon and Helena J MaierThe Pirbright Institute, Ash Road, Pirbright, Surrey, GU24 0NF

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Infectious bronchitis virus (IBV), an avian gammacoronavirus, is an important pathogen in commercial chicken populations causing significant economic losses to the UK poultry industry. The virus initially infects the respiratory tract and can spread to the kidneys and uro-genital tract. Infection leads to decreased egg production and quality, growth retardation and higher mortality rate due to secondary infections. During replication in the host cell, IBV has been shown to induce the formation of zippered ER and tethered vesicles known as spherules. For related viruses, rearrangement of cellular membranes allows assembly of viral RNA synthesis machinery and formation of the replication/transcription complex (RTC). However, the location of RTC assembly and viral RNA synthesis is unknown for IBV or any coronavirus. Historically, dsRNA has been used as a marker for sites of coronaviral RNA synthesis. However, the role of dsRNA during replication of coronaviruses remains controversial. Previously, immunofluorescence labelling showed that IBV associated dsRNA and viral RNA dependent RNA polymerase, nsp12, do not colocalise in infected cells. Here we have used bioimaging techniques to further characterise the cellular location of IBV RTCs and viral RNA synthesis. Initially we characterised the location of IBV associated dsRNA and nsp12 and colocalisation with markers for various cellular organelles. We also demonstrated that both dsRNA and nsp12 are located within membrane protected compartments by comparing immunofluorescence labelling of cells permeabilised with different detergents. Finally, nascent RNA synthesis has been visualised using incorporation of uridine analogues.

S13/P3

Variation of membrane rearrangements induced by M41 strain of infectious bronchitis virus

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Positive-strand RNA (+RNA) viruses rearrange cellular membranes during the virus lifecycle to create replication organelles, thought to allow more efficient viral RNA synthesis. Economically important avian coronavirus, infectious bronchitis virus (IBV), was shown to induce formation of conserved double membrane vesicles induced by all coronaviruses. In addition, IBV caused regions of ER to zipper together and open-necked spherules, strongly resembling spherules induced by other +RNA viruses, were shown to be tethered to the zippered ER. This previous work was performed using an apathogenic, highly laboratory adapted virus strain, Beau-R. In our recent work, we have shown that in cell culture, a pathogenic laboratory strain, M41, induced very low numbers of spherules. In spite of this, overall virus replication was unaffected with comparable accumulation of viral RNA and peak virus titres between Beau-R and M41. A more detailed comparison of a range of apathogenic and pathogenic vaccine and field isolates in *ex vivo* tracheal organ culture demonstrated that M41 was the only viral strain studied to have a low spherule phenotype. Therefore, although it appears that for most IBV strains spherules play a role during virus replication, coronavirus replicative apparatus clearly has the plasticity to function in different structural contexts.

S13/P4

The Effect of pH and Receptor Glycosylation on the Structure and Binding of Feline Calicivirus.

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The Caliciviridae are a group of small, non enveloped viruses with a positive sense, single stranded RNA genome. The most important of the Caliciviridae are the Noroviruses. These are, however, unable to be grown in cell culture and so Feline Calicivirus (FCV) is often used as a tractable model. FCV causes respiratory illness and stomatitis in cats. The receptor for FCV is the feline junctional adhesion molecule A (fJAM-A). Cryo-electron microscopy and three dimensional image reconstruction were used to determine the structure of FCV, decorated and undecorated with fJAM-A, at a range of pH from 4 to 7. At pH7, a conformational change is seen on the receptor decorated virion. We hypothesise that this is the first stage of virus uncoating. The structure of FCV at pH5 showed no significant conformational changes from the neutral pH counterpart. For fJAM-A decorated virus at pH 4 disassembly was seen, whereas undecorated virus showed no loss of integrity or structural change. We also investigated the oligomerisation state of the receptor and tested the effect of the glycosylation state of fJAM-A on virus attachment. We found that fJAM-A glycosylation did not affect virus binding in vitro. However, preliminary data suggests a difference in kD between the interaction of FCV with glycosylated or desialylated fJAM-A, although different biochemical techniques must be employed to investigate this further. This structural information alongside molecular virological data may contribute to the development of a more effective vaccine against FCV.

S13/P5

Residue 146 in domain I of hepatitis C virus NS5A regulates hyperphosphorylation and RNA replication in a genotype-dependent manner.

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Hepatitis C virus NS5A protein is a highly phosphorylated protein with critical, albeit poorly defined, roles in both virus genome replication and virion assembly. NS5A manifests as two phosphorylated species - basally and hyperphosphorylated - that migrate with apparent molecular masses of 56 and 58kDa respectively on SDS-PAGE. The roles of phosphorylation in regulating the various functions of NS5A are only just beginning to be unravelled. In this regard, we previously reported that phosphorylation of S146 in the N-terminal domain I of NS5A in the cell culture infectious genotype 2a clone, JFH-1, had no effect on RNA replication but inhibited hyperphosphorylation (Ross-Thriepland & Harris, J.Virol. 2014). Here we investigate the role of the corresponding residue (A146) in the genotype 1b non-culture adapted Con1 isolate. Mutation of this residue to aspartate (A146D: phosphomimetic) resulted in a 10-fold reduction in replication, whereas A146S (ie mimicking the corresponding JFH-1 residue) had no phenotype. Impaired virus replication of the mutant A146D was associated with NS5A levels and hyperphosphorylation and both mutants altered the distribution of NS5A, restricting it to a perinuclear region. We conclude that the inhibition of hyperphosphorylation by residue 146 is conserved between genotypes 1b/2a. However, in the non-culture adapted Con1 isolate, unlike in both JFH-1 and culture-adapted isolates, a loss of hyperphosphorylation correlates with a reduction in replication, suggesting that caution needs to be exercised when extrapolating data between genotypes.

S13/P6

Suppression of MHV infectivity by virus proteins and dominant negative mutants

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Abstract

Coronaviruses are enveloped positive sense, pathogenic RNA virus that infect many species of animals, including humans. Coronaviruses encode about 30 proteins that play specific, and often essential, roles in viral replication and assembly. In order to develop new protein antivirals and better understand how the viral proteins interact, we have used mutagenic PCR and virus-based selection to identify mutated proteins that act as dominant negative inhibitors of some aspect of coronavirus replication. This directed evolution approach resulted in both mutations with positive and negative effects on virus growth.

The goal of this project is to develop a small protein inhibitor from mutated coronavirus genes components that have been selected by directed *in vitro* evolution. In this study, structural proteins such as (N, M genes), non structural proteins (5,6,7,8,9,16) from gene1 and part of nsp (Y from C terminal of nsp3, PLpro, RdRp from C-terminal and N-terminal of nsp12 of Murine hepatitis virus strain A59 (MHV-A59) were cloned in pTriEx1.1 vector and expressed in *E.Coli* BL21 cells to allow purification of proteins of interest. SUMOSTAR (small ubiquitin-like modifier) fusion technology was used to enhance protein expression in eukaryotic systems. We tested ability of virus-expressed proteins in eukaryotic cells to interfere with virus infection.

In the next phase of the project, we will examine the effects of non- mutated and mutated proteins on virus replication based on their ability to protect cells from virus-induced cytopathic effects.

S13/P7

Development of novel Adhiron based biosensors for the detection of viral proteins

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is an infection of swine with a substantial economic impact worldwide. This project aims to produce a novel lateral flow biosensor which is able to detect the presence of PRRSV nucleocapsid protein in a sample collected directly from an infected animal. The device is based upon a current pregnancy test biosensor, where the antibody components will be replaced with the novel non antibody binding proteins, Adhirons. Adhirons are smaller and more stable than antibodies and are a small cystatin based protein scaffold, with two variable loop regions each with 9 randomised amino acids. Adhirons with high specificity against target proteins can be raised using phage display techniques; in this case the target is the nucleocapsid protein from a high and a low pathogenic strain of PRRSV.

Adhirons specific to these target proteins are now being used in the development of lateral flow devices to investigate the suitability and sensitivity of these reagents in this platform. Identification of pairs of Adhirons which can recognise the target proteins is required for the functioning of a lateral flow platform and this was carried out using Adhirons immobilised to both gold nanoparticles and magnetic beads in a pulldown assay. Suitable pairs of Adhirons for each target were then integrated into the lateral flow device by immobilisation onto a nitrocellulose membrane or gold nanoparticles as dipstick tests. The sensitivity of the reagents in detecting viral protein in a range of media and at varying concentrations was also investigated.

S13/P8

Dynamic interactions between *trans*-activating factors and structured RNA during dengue virus replication

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Dengue virus (DENV) is one of the most prominent arthropod borne viral infections in the world. However, there is no specific anti-viral therapy or clinically approved vaccine available. Control of DENV replication involves *cis*-acting RNA structures, which are distributed throughout the DENV genome and undergo a range of RNA-protein and higher order RNA-RNA interactions. Moreover, the DENV genome is dynamic, forming essential linear and circular conformations, via the interaction of cyclisation sequences at either end of the genome. Mechanisms controlling the spatial and temporal regulation of these *cis*-acting RNA elements and genomic conformational changes remain to be elucidated. Both the function and dynamics of the RNA-RNA and RNA-protein interactions, during different stages of replication remain unclear. Combining structural methods (including a range of SHAPE mapping and NMR techniques) and reverse genetic analysis, we are investigating the dynamics of interactions between alternative DENV genome conformations/ RNA elements, the viral polymerase (NS5) and other elements of the replication complex. As well as dissecting their role during early virus replication, analysis may provide proof of principle for therapeutic targeting of dynamic DENV RNA structural interactions, an interesting avenue for direct acting anti-viral research.

S13/P9

Exploring the structure and function of the non-primate hepacivirus 5'UTR

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Non-primate hepacivirus (NPHV) is a newly discovered virus in the genus hepacivirus, identified in domestic horses. It is the most closely related virus to hepatitis C virus (HCV), yet it does not appear to be associated with disease in horses. It has the potential therefore to facilitate comparative analysis of the two viruses in order to identify mechanisms of pathogenicity. However there is currently no system available to study the replication of NPHV.

Internal ribosome entry sites (IRES) are highly structured regions of RNA which facilitate cap independent initiation of translation and are utilised by a wide range of viruses from a variety of genus and families. The NPHV 5'UTR shares a high degree of structural similarity with the HCV 5'UTR and, like its HCV counterpart, possesses IRES function. To further elucidate the necessary structural elements of the NPHV IRES, mutational analysis has been conducted where predicted RNA structures have been systematically deleted. This analysis was conducted using a bicistronic reporter system where renilla luciferase is translated in a cap dependent fashion and firefly luciferase is translated by the NPHV IRES, allowing the two to be compared to determine IRES efficiency. This has revealed that the first two stem loops of the NPHV 5'UTR are not required for translation; however their presence does enhance IRES function. A number of cell lines stably over expressing miR122, which is essential for the HCV lifecycle, have been established to elucidate the role of the putative miR122 binding sites present in the NPHV IRES.

S13/P10

Depletion of the deubiquitylase enzyme MSYM1 leads to a decrease in alphavirus replication

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The mosquito-borne alphavirus Chikungunya virus (CHIKV) can lead to a severe debilitating arthralgia in infected humans. Originally identified in Tanzania in 1952, CHIKV is present throughout South-East Asia, has been seen in Europe, and is now widespread throughout the Americas. Currently there are no licensed vaccines or antivirals. Using the less pathogenic model alphavirus, Semliki Forest Virus (SFV) we have initiated studies investigating the interaction of alphaviruses with the ubiquitin proteasome system, in particular the role of deubiquitylase (DUB) enzymes. Using a siRNA library containing pools of four siRNAs for 92 known or predicted DUBs, we identified that knockdown of MYSM1 in HeLa cells lead to an increase in cell viability after SFV infection, suggestive of a decrease in virus replication. Deconvolution of the siRNA pool confirmed that when employed individually, all four MYSM1 siRNAs caused the same increase in cell viability after SFV infection. Further experiments showed that knockdown of MYSM1 lead to a reduction in both SFV plaque formation and release of SFV in the supernatant. This correlated to a decrease in viral RNA levels within infected cells as determined by RT-PCR. These studies are being extended to investigate the role of MYSM1 in CHIKV infection, and to further characterise the function of MYSM1 during virus infection. DUBs are receiving a great deal of attention as targets for therapeutic intervention in areas such as cancer, and this could be extended to viral infections where manipulation of the activity of MYSM1 may be a potential target for anti-viral treatment.

S13/P11

Evidence of absence for a proposed conformational change implicated in the translation/replication switch of HCV

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Replication of positive-strand RNA viruses requires that 'early' genomes act as templates for the mutually incompatible events of translation and replication, at some point switching from the former to the latter. The core protein of hepatitis C virus encodes a conserved stem-loop structure (designated SL87) predicted to undergo a conformational change potentially involved in modulating this switch. A sequence in the 5' base stem of SL87 is predicted to form a long-range RNA-RNA association (LRA) with complementary sequences located at one of two miR122 binding sites (site 1 and 2) within the 5'UTR. In an 'open' conformation SL87 forms and miR122 can bind to site 1 and 2, thus favouring translation given the known effects of miR122 binding. In a 'closed' conformation, the 5' base stem of SL87 interacts with the 5'UTR via the LRA and competes with miR122 for binding at site 1. This is predicted to form a more compact structure favouring replication. Using reverse genetics we analysed several mutants designed to disrupt, or favour, formation of SL87 or the proposed LRA. In parallel, RNA structure mapping by SHAPE analysis found limited evidence to support existence of the predicted 'closed' conformation in *in vitro* systems. Furthermore, we observed only insignificant changes in translation and replication levels when compared to wild type virus, in mutants designed to test both conformations. We propose SL87 does not undergo the predicted conformational change but do not rule out the possibility that SL87 has an alternative role to play in regulating translation/replication.

S13/P12

Increased identification of noroviral infections using a novel TaqMan Array Card assay.

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Gastroenteritis is responsible for over one billion cases yearly worldwide, which result in two million deaths. Detection of the gastroenteric aetiological agents is performed by a plethora of assays that may bias the comparison of the results obtained between different tests and laboratories. The TaqMan Array Card (TAC) provides a RT-qPCR assay that is suitable for the fast detection of multiple targets.

Residual stool specimens, one per patient, were obtained from the Diagnostic Laboratory of the Addenbrooke's hospital (Cambridge, UK). The specimens were bead-beated with MagNA Lyser (Roche), extracted using SymphonyDP (QIAgen) and amplified by the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher) on customized TAC plates (Thermo Fisher).

The present study analysed 773 patients for a panel of 25 gastrointestinal pathogens. The TAC assay identified 136 (17.6%) and 27 (3.5%) patients with single and multiple infections, respectively, that were missed by the routine testing. Conversely, the routine testing detected five (3.1%) and one (0.6%) patients

with single and multiple infections, respectively, that were missed by the TAC assay. Norovirus genotype GII (NoV-G.II) was the most common infectious agent (n=144), followed by Dientamoeba fragilis (n=35) and Yersinia enterocolitica (n=34). NoV-G.II was identified in co-infection in particular with Dientamoeba fragilis (n=9), Clostridium difficile (n=7) and Yersinia enterocolitica (n=6).

The present study demonstrated the potential of TAC as an effective screening tool with increased sensitivity and pathogen coverage than the routine testing. Using this assay it was possible to identify noroviral infections that would have been missed otherwise.

S13/P13

Identification of antibodies involved in natural clearance of GBV-B through development of a virus neutralisation assay.

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Hepatitis C virus (HCV) infection is a growing public health concern with over 150 million people chronically infected with HCV worldwide and approximately 500,000 deaths every year from HCV-related liver diseases. There is currently no vaccine, and treatment outcomes are highly variable; progress is hampered by the difficulty in culturing the virus in vitro and lack of a reliable animal model. Recently, another hepacivirus, GBV-B, has been used as a surrogate model for HCV, as the two viruses have a close phylogenetic relationship. HCV infection has a high propensity to become chronic, whilst GBV-B is naturally cleared in its New World monkey (e.g. tamarin) host, thereby providing a model to study viral clearance.

To investigate the humoral immune response, a GBV-B neutralisation assay is required. Based on a method used for HCV, we aim to produce a pseudotyped virus (PsV) expressing GBV-B envelope proteins that can infect a suitable cell line. Archived GBV-infected tamarin sera will later be tested in this system. Neutralising antibodies found through this model could be epitope mapped, and potentially incorporated into vaccine design strategies.

S13/P14

Enterovirus E empty capsids: Dead-end product or viral building block?

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Enterovirus-E (formerly bovine enterovirus) is an enterovirus of the picornaviridae. It causes ubiquitous symptomless infection in cattle and is harmless to humans. This study aims to determine if RNA-free empty capsids produced during infection are functional intermediates in virion assembly or surplus dead-end products. During viral assembly, 5S protomers (VP0, VP1, VP3) form 14S pentamers $(VP0, VP1, VP3)_5$. The provirion is formed from pentamers $[(VP0, VP1, VP3)_5]_{12}$, that rearrange to form mature virions through VP0 cleavage to VP2 and VP4. Pentamers also form 80S RNA-free empty capsids $[(VP0, VP1, VP3)_5]_{12}$. Li *et al* (2012) proposed that empty capsids assembled *in vitro* are too stable to be direct precursors of virions and are dead-end products. The ratio of empty capsids to mature viruses was increased by inhibiting RNA synthesis with guanidine hydrochloride (GuHCl). BHK cells were infected with BEV at MOI=10, GuHCl was added at 4 hours post-infection for maximum empty capsid production. Radiolabelled empty capsids and mature viruses were purified on sucrose density gradients, and their ratios determined by scintillation counting. Pulse-chase experiments showed that labelled empty particles chase into full virions once GuHCl inhibition was lifted. The stability of the 80S empty capsids was studied by TEM. At 37 °C empty capsids dissociate to pentamers within 24 hours, whereas mature BEV capsids are stable for more than 30 minutes at 60 °C. This study demonstrates that the empty capsids assembled *in vivo* can be recycled into mature virions and must therefore differ from those reassembled *in vitro*.

S13/P15

Investigation into glycosylation of the spike glycoprotein of infectious bronchitis virus

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Infectious bronchitis virus (IBV) is a gammacoronavirus that infects chickens. It causes major losses in the poultry industry due to reduced meat quality and egg production. Although vaccines against the virus exist, protection is often short-lived and cross-protection between serotypes is poor. IBV has four structural proteins known as the nucleocapsid, envelope, membrane and spike proteins. The spike protein is responsible for attachment to host cells and fusion of viral and cellular membranes. N-linked glycosylation is defined as the attachment of a glycan to a protein via the asparagine (Asn) in the amino acid sequence Asn-X-Ser/Thr, where X is any amino acid apart from proline. N-linked glycosylation is the most common form of glycosylation

among viral proteins and has been shown to affect many aspects of the virus including tropism, infectivity and immunogenicity. Like many other viral proteins, the IBV spike protein is highly glycosylated and the extent of glycosylation varies between strains. As different strains exhibit different tropisms, symptoms and pathogenicities, it is possible that glycosylation influences some of these viral traits. The spike protein has been studied to identify potential glycosylation sites in the sequences of two strains of IBV (M41-CK and Beau-R). These sites will be mutated and reverse genetics will be used to generate recombinant viruses with modified spike proteins. The characteristics of these viruses will be investigated to see whether the elimination of selected glycosylation sites has impacted the virus in any way. This may provide a basis for more rational vaccine development against IBV.

S13/P16

Norovirus evolution: understanding and characterising the emergence of novel strains in the population

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Noroviruses (NoVs) are globally distributed, affect all age groups and place a significant burden upon health services. To control NoV infection and transmission it is important to understand the emergence of novel variants during shedding. Low frequency novel variants can be detected with in-depth Next-Generation Sequencing (NGS) data covering the whole genome. Clinical samples positive for NoV are complex, and prior to NGS, often require enrichment or PCR to overcome low viral loads.

In a pilot experiment, two different capture intermediates were used, porcine gastric mucin coated magnetic beads (PGM-MBs) or oligo(dT) cellulose, to increase the yield of NoV RNA available for downstream processing. PGM-MB capture conditions were optimized to increase the yield of GII NoV, and pooling before extraction meant it was possible to further concentrate virus 10 fold. The PGM-MB protocol was applied to longitudinal patient samples with acute illness.

For the investigation of virus diversity, methods of enrichment were assessed prior to next generation sequencing. The number of reads mapped to a NoV reference genome was comparable between enriched samples and those receiving further PCR amplification. By using NoV enrichment methods exploiting NoV binding to mucins, NoV genomes could be sequenced whilst avoiding the need for any strain-specific amplification. The NGS data from the pilot and longitudinal samples will be processed further to assess genome coverage, virus diversity and the presence of minor variants.

S13/P17

Longitudinal whole genome sequencing of GBV-B in acute infection

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GBV-B is a virus in the flaviviridae family distinct from but phylogenetically closely related to Hepatitis C virus (HCV). GBV-B has a similar genome length and organisation to HCV, with functional homology in defined regions. Using the surrogate GBV-B/tamarin model of HCV to study acute infection we find that some animals display a typical acute viraemia and others have prolonged infections. Understanding the impact of viral variation on the peripheral viral dynamics - particularly clearance - could inform antiviral design. Whole-genome deep sequencing of virus recovered from typically infected tamarins revealed contrasting structural protein conservation and non-structural protein diversity, primarily in the NS5A protein c-terminus. Mapping the position of GBV-B NS5A residue changes reveals an analogous pattern to those which appear in HCV NS5A. Modelling their effects on predicted secondary structures highlights changes in key regions, indicative of their functional effects. In tamarins with prolonged infection, major variants in the envelope protein have been observed alongside non-structural changes. Continuing investigations aim to analyse viral variation upon secondary challenge and from liver samples, to better understand the role of sequence variation on success of clearance of virus which may inform immunotherapeutic development.

S13/P18

Functional analysis of the Chikungunya virus nsP3 macro-domain

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Chikungunya virus (CHIKV) is an Alphavirus, first detected in Tanzania in 1952 but more recently re-emerging to spread globally. Virus infection presents as fever, rash and debilitating joint pain that can persist for years. CHIKV is a small, enveloped, positive-sense RNA virus. Of four non-structural proteins (nsP1-4) required for genome replication, nsP3 is the least well understood. The protein comprises three domains, the structure of the N-terminal of these has been determined, defining it as a macro-domain. These domains are present in a number of +ve-RNA viruses and in many cellular proteins - they bind RNA and ADP-ribose. However, their role(s) in the virus lifecycle remain unclear.

We aim to determine the function of the CHIKV nsP3 macro-domain. Based on the structure of the CHIKV nsP3 macro-domain we have mutated a range of key residues implicated in RNA or ADP-ribose binding. Phenotypic analysis of these mutants has revealed a key role for the macro-domain in replication of the

CHIKV replicon. One particular mutant, D10A, consistently reduces RNA replication when compared to wildtype in both mammalian and mosquito cell lines. Interesting, the phenotype of this mutant varies between cell lines. We are also investigating potential interactions between the nsP3 macro domain and cellular proteins. Alongside the cellular work, we are analysing properties of the macro domain (both wildtype and mutants) in vitro, including the binding to RNA and ADP-ribose, in order to correlate these to phenotype in virus genome replication.

S13/P19

Functional Analysis of The RNA Secondary Structures at The 3' termini of Murine Norovirus (MNV) Genomic RNA

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Human noroviruses (HuNoV) from the *Caliciviridae* family are considered as the major cause of non-bacterial acute gastroenteritis worldwide. However, our understanding of these important viruses is still limited due to the difficulties of growing HuNoV in cell culture. Murine norovirus (MNV) has been widely used as a model to understand the molecular mechanisms of human norovirus. The termini of the MNV genomic and subgenomic RNAs contain highly conserved RNA secondary structures. These RNA secondary structures have been shown to interact with various host factors, and thus play important roles in the virus life cycle. The 3' end of the MNV genome contains three predicted stem-loop structures (SL1-3). SL1 is the largest, typically 138 ntds, and is within the VP2 coding region. While SL2 and SL3 have been studied in detail, SL1 remains largely uncharacterized. In this study, we aimed to use reverse genetics system to introduce synonymous mutations to disrupt the stability of SL1, and examine the effect on virus replication. Our data indicate that the stability of SL1 is critical for viral replication.

S13/P20

Characterization of the function of the norovirus NS1/2 and NS4 proteins

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Human Norovirus (HuNoV) is a major cause of gastroenteritis worldwide, and of mortality in the developing world. In addition, HuNoV can establish persistent infections in immunocompromised hosts, which can result in both chronic disease and prolonged virus shedding. Currently, there are no effective therapeutics for norovirus disease and HuNoV is particularly difficult to study, due to the lack of a robust cell culture system. However, murine norovirus is a widely used model, for which animal, cell culture, and reverse genetics systems exist. The functions of murine norovirus proteins NS1/2 and NS4 currently remain unclear, although it is speculated that they may be involved in recruiting cellular membranes to form the viral replication complex. In addition, certain mutations in NS1/2 are known to be involved in establishment of persistent infection confirming a role for NS1/2 in norovirus pathogenesis. In order to better understand the roles of NS1/2 and NS4 in the virus lifecycle, we are employing several proteomics-based approaches to identify both viral and host cell protein interaction partners of these proteins.

S13/P21

RNA structure and function in Middle Eastern Respiratory Syndrome Coronavirus

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We have discovered the sequence of a new, highly divergent virus that has tentatively been named Abyssovirus in a pool of sequenced intracellular RNA from a metazoan. Based on comparison of the genomic organization and replicase subunits, Abyssovirus appears to belong to a new family of Nidoviruses. The aim of the present project is to validate the sequence data by studying the function of the predicted unusual translational stop-start signal located in the replicase gene, and to express two predicted viral proteases and test their catalytic activity in *E. coli* and mammalian expression systems. The translational stop-start signal was found to allow ribosomes to read through the natural stop codon with approximately 5% efficiency in the expression systems tested. Additionally, both abyssovirus proteases showed evidence of cleavage of fragments from both ends of the expressed protease, as is typical for polyprotein-embedded Nidovirus proteases. These results suggested that the Abyssovirus RNA sequence encodes functional biological parts, and therefore that the RNA sequence likely does represent a new type of virus with some Nidovirus-like features and an Abyssovirus-specific mechanism to translate the second half of the replicase open reading frame.

S13/P22

Picornavirus capsid protein VP4: mechanism for membrane permeability and potential target for neutralising antibodies

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Picornaviruses such as foot-and-mouth disease virus (FMDV) and human rhinovirus (HRV) are the infectious agents of economically important diseases affecting humans and livestock. VP4 is a small and highly conserved internal capsid protein, which is released during virus entry. Work from our lab and others has previously demonstrated VP4 plays a role in virus entry by forming size-selective pores in membranes, facilitating

genome entry. In addition, the N-terminus of VP4 is transiently exposed due to a mechanism called virus breathing. Consequently, antibodies against VP4 may be able to facilitate a protective immune response. The aim of our work is to characterise the pore formation and genome release function of VP4 and to determine if antibodies against VP4 can inhibit membrane interactions of recombinant VP4 with model membranes and neutralise virus infectivity. Our results suggest that both termini of FMDV VP4 are able to interact with model membranes and induce permeability. This differs from HRV VP4 where only the N terminus induces membrane permeability and forms size-selective pores. This suggests alternative mechanisms for transmembrane pore formation in FMDV and HRV. VP4 sequences will be presented on hepatitis B core virus like particles and the neutralising antibodies generated against VP4 will be investigated.

S13/P23

Further design of thermostable poliovirus VLPs using deep-sequencing

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We previously reported the design of thermostable type 2 poliovirus empty capsids (VLPs), suitable for vaccine development, using a strategy based on deep-sequencing information. The same process has now successfully been applied to the design of type 1 VLPs. Potential stabilising mutations were identified and then incorporated in several combinations into full-length type 1 polio clones. VLPs were generated and assessed for stability. The most promising candidates were tested for immunogenicity and seroprotection *in vivo* and found to be at least as potent as the current inactivated vaccine reference preparation.

A recombinant polio VLP vaccine, which would not require any live virus in its production, would be ideal for the post-eradication, high containment world.

S13/P24

The effects of deletions within FMDV 3A on replication

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Foot-and-mouth disease virus causes a highly infectious disease in cloven hoofed livestock species and outbreaks have a high economic impact through loss of trade, animals and productivity. Better understanding of viral replication can lead to improved vaccines and control of the disease.

The FMDV non-structural protein 3A is much longer than in other picornaviruses, 153 amino acids compared to just 87 amino acids in poliovirus. Deletions in 3A of amino acids 93-102 and 133-143 have previously been discovered and attenuate the virus's ability to infect cattle and grow in bovine cells *in vitro*, while remaining infectious to pigs and capable of growing in porcine cells (Pacheco et al., 2003). This implies FMDV 3A may have a role in determining viral host range.

This work investigates effects of a large deletion in 3A in the C terminal region (amino acids 81-137), on replication. The same deletion was made in the 3A protein of an FMDV replicon with a GFP reporter gene. Replication in BHK and porcine cells was unaffected but was completely attenuated in MDBK cells. Trans-complementation assays were used to test if replication could be restored in MDBK cells, by providing WT 3A in *trans*, in the form of a second replicon with a mCherry reporter gene. Results showed an increase in GFP intensity in MDBK cells when the two replicons were co-transfected. This suggests full length 3A is required for viral replication in bovine cells and that it can be trans-complemented effectively to restore replication.

S13/P25

Role of a single nucleotide non-synonymous mutation in the foot-and-mouth disease virus non-structural protein 2C, during adaptation to a new cellular host *in vitro*

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High mutation rates of RNA viruses result in dynamic viral population structures which determine fitness of these viruses and allow them to rapidly overcome selective constraints. While virus population structure has been studied for several RNA viruses, it is still unclear how quasispecies dynamics may affect adaptation of a virus to a new host.

Foot-and-mouth disease virus (FMDV) is a positive-sense RNA virus of the family *Picornaviridae*. We previously described a study in which a hamster cell line (BHK21)-adapted virus was in turn adapted to growth in a bovine cell line (a natural cellular host for this virus). After serial passage in the bovine cell line, adaptation was observed as a doubling of the rate of growth, which correlated with appearance of a non-synonymous mutation in the non-structural protein 2C. Changes in 2C upon adaptation to a new host have been described before for other picornaviruses. Interestingly, despite the observed change in growth phenotype, the mutation remained a minority component of the viral population (43%). In the current work, trying to understand how minority populations affect adaptation of FMDV, we engineered the parental FMDV strain with the same 2C mutation and studied its replication in the bovine cell line. This provided a system to determine whether this mutation alone affects FMDV phenotype, or whether more complex alterations in population structure are required for FMDV adaptation.

S13/P26

Discovery of Novel Alphacoronaviruses in European Rodents and Shrews

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Coronaviruses (CoVs) infect a plethora of both animals and birds. The recent emergence of the novel SARS and MERS coronaviruses in humans and porcine epidemic diarrhoea virus (PEDV) in pigs indicates that coronaviruses have significant zoonotic and epi-zoonotic potential. In humans, coronaviruses are associated with respiratory disease. In mammals and birds, coronaviruses have been associated with enteric and respiratory diseases as well as hepatitis and neurological disorders. To date, with the exception of the murine hepatitis virus (MHV), there have been few reports of coronaviruses in European rodents. In this study, 813 European rodents encompassing seven different species were screened for alphacoronaviruses using degenerate PCR. Four novel alphacoronaviruses were detected in the species Rattus norvegicus, Microtus agrestis, Sorex araneus and Myodes glareolus. These, together with the recently described Lucheng virus found in China, form a distinct clade in the coronavirus phylogeny. This study has shown the first evidence of alphacoronaviruses present in European rodents and also highlights that Eurasian rodent alphacoronaviruses described to date form a single clade within this genus. Coronavirus infection of rodents appears widespread, thus these animals may pose a threat for cross-species transmission to humans and/or other animals.

S13/P27

Unravelling the Pseudoknots: The Role of Pseudoknots in FMDV Replication

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Foot-and-mouth disease virus (FMDV) is a positive-sense single-stranded RNA virus belonging to the Picornaviridae family. FMDV is the causative agent of foot-and-mouth disease, a disease affecting cloven hooved animals, outbreaks of which have a devastating impact on the economy. FMDV is unique amongst picornaviruses due to its large 5' untranslated region (5' UTR) of over 1300 bases comprising of a number of conserved RNA structures. One of these features is a series of pseudoknots at the 3' end of the poly-C tract, a location which is unique to FMDV. Pseudoknots are found in the non-translated region across a broad spectrum of viruses and have diverse functions with roles in frameshifting, translation and splicing as well as viral replication. Pseudoknots are present in other picornaviruses such as cardioviruses, where they are present at the 5' of the poly C tract and have been implicated in viral replication. Interestingly the number of pseudoknots between FMDV strains is variable, with isolates containing 2-4 tandemly repeated sequences and the role of FMDV pseudoknots is yet to be established. We have created several mutants by deleting pseudoknots and monitored their impact on replication in the FMDV replicon to derive a model of pseudoknot function.

S13/P28

Differential gene expression in an *Ixodes ricinus* tick cell line following infection with tick-borne viral and bacterial human pathogens, and the detection of a number of host genes associated with restriction of infection

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The emergence and expansion of tick-borne diseases in Europe, Asia and North America has been observed in recent years. The flavivirus tick-borne encephalitis virus (TBEV) is responsible for the increasing number of cases of tick-borne encephalitis (TBE) detected in humans throughout Europe and Asia, and is transmitted by *Ixodes ricinus* (*I. ricinus*) ticks. *Anaplasma phagocytophilum* (*A. phagocytophilum*) is considered an emerging zoonotic bacterium, is transmitted by *I. ricinus* ticks in Europe and *I. scapularis* in the United States, and causes human, canine or equine granulocytic anaplasmosis and tick-borne fever in ruminants. Differential gene expression in an I. ricinus cell line infected with these tick-borne pathogens was assessed using a systems biology approach, incorporating next generation sequencing (NGS) and subsequent transcriptomic analysis. Both pathogens induced differential gene expression in tick cells, including genes involved with apoptotic processes and immune function, including the gene for heat shock protein 70 (HSP 70), which inhibits apoptosis. In particular, genes associated with the restriction of infection within the tick cell have been identified, either via the immune response, or with a direct anti-pathogen function, to enhance cell survival following infection. These data provide important information about host-pathogen interactions, which may potentially be applied to the design of improved therapies or infection prevention strategies.

S13/P29

Investigating changes of cancer stem cell marker expression caused by HCV infection

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Chronic hepatitis C virus (HCV) infection is associated with a high risk for progressive hepatic fibrosis, cirrhosis and ultimately the development of hepatocellular carcinoma (HCC). However, the link between HCV and HCC is poorly understood and controversy exists as to whether the virus plays a direct oncogenic role; dogma attributes HCC development to increased cell turnover and the inflammatory microenvironment within the infected liver. We hypothesised that HCV might increase expression of cellular proteins associated with a poor HCC prognosis. Interestingly, HCV replicons induced a significant increase in the expression of CD24, a GPI-anchored protein capable of activating Src-related kinases, antagonising p53 function, and which is heavily implicated in the self-renewal ability of HCC cancer initiating cells. Accordingly, Huh7 cell sub-populations sorted for high/low CD24 expression displayed enhanced, or greatly diminished tumourigenicity in SCID mice compared with parental Huh7 cells, respectively. Interestingly, induction of CD24 expression was dependent upon the ability of the HCV NS5A protein to interact with cellular SH3-containing proteins, and upregulation was also observable within sorted CD24lo Huh7 populations, which have previously been shown not to be capable of such activation in response to other stimuli. We are currently investigating the mechanism by which CD24 is upregulated, with focus on a STAT3/HIF1a centered cellular pathway, both of which are key factors in HCC pathogenesis. These investigations are being conducted with direct reference to effects upon cellular proliferation, tumourigenicity, and differentiation status.

S13/P30

Foot-and-mouth disease virus conserved internal capsid components as targets for neutralising antibodies.Amin S. Asfor, Anusha Panjwani, Krupali Parekh, Mana Mahapatra, Satya Parida, Toby Tuthill.The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 0NF

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Foot-and-mouth disease virus (FMDV) is responsible for a highly contagious disease of livestock that causes significant financial losses. The FMDV capsid is assembled from 60 copies of four structural proteins (VP1, VP2, VP3 and VP4). VP1, VP2 and VP3 all contribute to the exposed surface of the capsid. VP4 and the N-terminus of VP2 are internal and are highly conserved among all FMDV serotypes. Studies with other related picornaviruses have shown that VP4 is transiently exposed in a process termed virus breathing and is completely externalised from the capsid to interact with membranes during cell entry.

To investigate the potential role of these internal capsid components as conserved B cell epitopes, sera from infected animals were screened for reactivity against peptides representing VP4 and the N-terminus of VP2. Antibodies were detected against peptides representing N-termini of both VP4 and VP2. Interestingly, a previously characterised neutralising monoclonal antibody reacted with both the VP4 and VP2 peptides. Cross reactivity with sera collected from animals vaccinated with all the 7 serotypes of FMDV was also observed. The results of this study suggest that capsid components thought to be internal may form a novel conserved surface-exposed epitope and may aid in the design of an epitope vaccine against all serotypes of foot-and-mouth disease.

S13/P31

Sequence-specific RNA recognition and its implications for viral assembly

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The assembly mechanisms of single-stranded, positive-sense RNA viruses have typically been thought of as dominated by protein-protein interactions. However, a wholly protein controlled assembly mechanism does not account for the observed selectivity of genome packaging seen *in vivo*[1]. RNA SELEX has identified up to 30 sequences in the genome of Satellite Tobacco Necrosis Virus-1 (STNV-1) that might bind specifically to cognate coat protein. These RNA sites, termed packaging signals (PSs), can form stem-loop structures, presenting an AXXA sequence in 4-, 5- or 6-nucleotide loops[2]. Using single molecule fluorescence correlation spectroscopy (smFCS), a technique that measures the hydrodynamic radius of a sample at single molecule concentrations, it has been demonstrated that these regions act co-operatively to promote correct CP-CP interactions for efficient formation of capsids. Using a region from the 5' end of the STNV-1 genome containing 5 of these sites (PS1-5) it has been shown that both relative spacing and loop sequence of these packaging signals affects particle assembly. The aptamer B3 shares 16/25 nucleotide sequence identity to the highest affinity site within this fragment and readily forms *T=1* virus like particles whereas a variant with a 4-uridine loop (B34U) is unable to assemble beyond capsomere suggesting that binding is sequence-specific. [3]. A library of B3 variants has been produced for determining the key recognition features of this RNA-protein interaction. Results suggest that AXXA is the optimal motif with the 3' adenine being more important for accurate particle assembly than the 5' adenine in the loop.

S13/P32

Detection of Dengue Viral RNA and Serotyping in NS1 Positive sera by PCR

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Dengue fever (DF) is a self-limited systemic viral infection which is transmitted among humans by mosquitoes. The rapidly spread of DF is a public health problem, with estimated of 2.5 billion people at risk of dengue viral infections and among theses about 975 million people are living in tropical and sub-tropical countries in Southeast Asia, the Pacific and the Americas. It is also estimated that about 50 million dengue infections occur globally every year and among these 500,000 are hospitalizations for dengue haemorrhagic fever (DHF).

The precise diagnosis of this infection helps in management of the patients and control this infection efficiently. This study is aimed to find out the frequency of antigen (NS1) detected in clinically suspected patients as well as serotyping of the dengue virus (DENV) from antigen positive samples. The samples were collected from Combined Military Hospital (CMH) Lahore, Pakistan and processed in Microbiology Department of University of Health Sciences Lahore Pakistan. Among a total 180 serum samples, 91 samples (50.5%) were positive for NS1 antigen. The PCR amplification of these antigen positive samples (n=91) for serotyping demonstrated that DENV 3 serotype (14.2%) were predominant followed by 8.8% of DENV 2 serotype and 3.3% were DENV 4 serotype. There was no case detected with DENV 1 serotype. Although, the sample size of present study is small but according to the results, DENV 3 is most common serotype prevalent in the area.

S14/P1

The Role of Gammaherpesviruses in the Pathogenesis of Malignant Catarrhal Fever in Cattle

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Malignant catarrhal fever (MCF) is an economically-important disease of cattle, bison, buffalo and deer worldwide. The disease is characterised by complicated pathogenesis in ungulate animals such as: ocular and nasal discharge, vasculitis, lymphoproliferation, and mucosal necrosis of the digestive system. It is frequently fatal. Currently, the causative agents belong members of the *Macavirus* genus within the gammaherpesvirus subfamily. The causative agent of MCF in the UK is ovine herpesvirus 2 (OvHV-2). Sheep are asymptomatic carriers in contrast to cattle, water buffalo and bison that succumb to MCF. Recently, our lab has shown that OvHV-2 can persist in non-diseased cattle suggesting that other factors, possibly other virus infections could be involved in the pathogenesis of MCF. Both bovine herpesvirus 6 (BoHV6) and the agent associated with sheep pulmonary adenomatosis , ovine herpesvirus 1 (OvHV-1) are closely-related to OvHV-2. We surmised that as well as OvHV-2, BoHV-6 and OvHV-1 might have a role to play in the pathogenesis of MCF. We therefore developed qPCR assays for these viruses in order to assess their potential involvement in MCF. Our results showed that as expected, BoHV-6 is endemic in cattle but not sheep. Interestingly, OvHV-1 is endemic in cattle as well as sheep. More importantly, although OvHV-1 was detected in a high proportion (58%; n=36) of normal cattle, it was not found in cattle with MCF (n=28). This suggests that OvHV-1 infection may in some way protect against the development of MCF in cattle.

S14/P2

Targeting host cell ion channels as potential anti-viral therapeutics against polyomavirus-associated nephropathy (PVAN).

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BK polyomavirus (BKPyV) is an emerging human pathogen that establishes a lifelong infection in kidney epithelial cells. BKPyV infects more than 80% of the general population worldwide. Immunocompromised patients, after renal transplantation, are potentially exposed to severe clinical complications such as polyomavirus-associated nephropathy (PVAN), which can ultimately lead to renal graft failure. With the ever increasing numbers of transplants and no direct acting anti-viral agents targeting BKPyV, clinicians are faced with the difficult choice of decreasing the dose of immunosuppressive drugs to battle virus infection or risking graft rejection. Therefore there is an urgent need to identify aspects of the viral lifecycle in order to find potential pathways that can be exploited. Ion channels play a critical role in kidney physiology, implicating them as candidate proteins required for BKPyV infection. Using pharmacological inhibitors of ion channels, we were able to block BKPyV replication in primary renal proximal tubular epithelial (RPTE) cells. Use of more specific inhibitor compounds indicated that the family of potassium channels was required for BKPyV production. Ongoing experiments are illuminating the pathways controlled by these channels during virus infection. These studies provide the first reported requirement for potassium channels in the BKPyV life cycle. This may be of great importance since potassium channels play crucial role in the control of renal function. Moreover, potassium channels are an emerging therapeutic target for many medical conditions and as such compounds that target this channel family may represent a novel strategy for developing therapeutics to treat PVAN.

S14/P3

Isolation and characterisation of Pectobacterium atrosepticum phages sensitive to two Type III bifunctional toxin-antitoxin/abortive infection systems

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The Type III toxin-antitoxin (TA) systems have been shown to be involved in abortive infection (Abi). Type III systems are thought to be altruistic bacterial defence machines that protect bacterial populations from their viral predators, bacteriophages, by precipitating precocious cellular suicide after initial infection. Our previous work showed that Pectobacterium phage, ΦM1, is highly sensitive to ToxINPa and, to a lesser degree, to TenpINPl - which represent two subfamilies of Type III TA systems. Characterisation of ΦM1 mutants that fail to activate either systems showed that all have mutations in ORF22 encoding a toxic protein of unknown function. We isolated a new phage (ΦRC10) from the River Cam in Cambridge during screening for Abi-sensitive phages. ΦRC10 was very sensitive to the TenpINPl system but insensitive to ToxINPa in terms of efficiency of plaque formation. Whole genome sequencing of ΦRC10 has shown that it is a highly similar to ΦM1. Additionally the data showed that it also contains a gene encoding an ORF22 protein that only differs by one amino-acid from that of ΦM1. Characterisation of all ΦRC10 escapes so far has revealed mutations in this phage's version of ORF22 suggesting both phages escape using the same mechanism and that a single amino acid change is able to alter the sensitivity to either TA systems.

S14/P4

Investigating the role of herpes simplex virus pUS3, pUL13, vhs, and ICP0 during the early stages of infection

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During the initiation of infection, viruses modify the cellular environment to usurp the cellular machinery and contend with the antiviral response. Enzymatic activity within the incoming virion permits a mechanism to immediately alter the host cell. Herpesvirus particles contain a proteinaceous tegument, and herpes simplex virus-1 (HSV-1) contains at least 4 enzymes. These are two kinases pUS3 and pUL13, the endoribonuclease vhs, and the ubiquitin ligase ICP0. Interestingly, none of these proteins are essential for virus viability in cell culture. We have generated deletion viruses for each of these tegument enzymes that are replication competent. In addition, we have also generated recombinant viruses expressing each of these viral enzymes tagged with fluorescent proteins to analyse their localisation within cells. To further characterise these proteins, we set out to produce monoclonal antibodies. Catalytically inactive mutants of these proteins were expressed in bacteria. While the kinases can be purified as full length proteins, vhs is readily cleaved into smaller fragments. The generation of deletion viruses and tools to track these enzymes from the tegument will allow for further investigation of the roles these proteins play during HSV-1 infection.

S14/P5

Analysis of environmental viunalikeviruses and their enterobacterial host ranges

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Viunalikeviruses are a recently defined genus of bacteriophages within the family *Myoviridiae* that infect a wide range of Enterobacteriaceae. These include clinically important organisms such as *Salmonella, Shigella, E. coli* O157:H7 and *Klebsiella*, as well as opportunistic human pathogens such as *Serratia* and the phytopathogen *Dickeya solani*. Viunalikeviruses have a classical T4-like morphology, but with tail-spike proteins attached to the baseplate instead of tail fibres. Current models suggest that the tail-spike proteins are key structural components involved in recognition of their specific host bacteria and likely facilitate host entry. Whilst the host range for specific viunalikeviruses is generally narrow, the bacterial receptor(s) for the respective tail spike proteins in different hosts has been understudied.

We have been investigating the role of bacterial surface components in viunalikevirus adsorption and penetration, and how this relates to viral host range. We have investigated a series of phages that infect Serratia and phages that infect *Dickeya*. Most of the phages were environmental viruses isolated through short-term enrichments. Some *Dickeya* phages were viunalikeviruses with narrow host ranges. After bacterial mutagenesis to score for resistance to the latter phages, we defined various genes associated with viral sensitivity. These genes were involved in a transport system, polysaccharide production and the cyclic-di-GMP economy of the host cell. Using gene fusions, we investigated the impacts of different carbon sources and other factors on the transcription of polysaccharide synthesis genes.

S14/P6

Interactome of Minor Capsid Proteins of BK Polyomavirus

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BK Polyomavirus (BKPyV) was firstly isolated from the urine sample of a kidney transplant patient suffering from ureteric stenosis. The infection which can undergo latent cycle in human kidney cells is asymptomatic. However, during prolonged immune suppression treatment in kidney transplant patients, BKPyV can cause polyomavirus-associated nephropathy (PVAN) and 80% of the cases lead to graft rejection. The viral capsid, which consists of major capsid protein, VP1 plays an important role in viral attachment to the host cells. However, the functions of the minor capsid proteins, VP2 and VP3 are poorly understood. A greater knowledge of the host binding partners of these capsid proteins may increase our understanding of their roles in the virus lifecycle. VP2 and VP3 fused to YFP and FLAG were generated. The subcellular localisation of the cloned proteins was compared to the GFP fusion VP2/3 as well as the native VP2/3 expressed by BKPyV in RPTEC and HEK293 cells. The VP2 and VP3 proteins were transfected into stable inducible cell line, HEK293 Flp-In™ TREx™ and were grown in Stable Isotope Labelling of Amino Acid in Cell (SILAC) media. The cell lysates were analysed using Mass Spectrometry (MS) and binding partners confirmed from MS analysis were further studied. By determining the binding partners of VP2 and VP3, we can get a better understanding of how important the interaction is in viral infection and survival in the host cells, and hopefully develop therapeutic treatment in the future.

S14/P7

Pro-apoptotic PRAF2 is a novel binding partner of the human papillomavirus E5 protein

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Human Papillomaviruses (HPV) infect cutaneous and mucosal epithelia and can lead to cancer development. HPV encodes for three oncoproteins E5, E6 and E7. In contrast to E6 and E7, very

little is known about the role of E5 in the productive stages of the virus life cycle and cancer progression. E5 is a small hydrophobic protein that is believed to reside in the ER and has recently been shown to interact with Bcl-xL/Bcl2 and modulate survival of cancer cells

Here we show by co-immunoprecipitation that a member of the prenylated rab acceptor family (PRA) 1 called PRAF2 interacts with E5 from a number of HPV types. PRAF2 is a 4 transmembrane domain protein related to the yeast YIP gene family that recently presents a candidate molecular target for therapeutic intervention in human cancer.

Further characterisation of PRAF2 in cervical epithelial C33A cells revealed a predominant membrane association by cell fractionation that localised to the endoplasmic reticulum and Golgi apparatus in immunofluorescence studies. Deletions of the N-terminal domain of PRAF2 demonstrated a potential role in ER export to the Golgi apparatus. PRAF2 localisation changes upon treatment with apoptotic stimuli. Further experiments showed PRAF 2 is a downstream target of BAX and that it may function in disrupting the pro-survival proteins Bcl-2 and Bcl-XL.

Further characterisation of PRAF2 interaction with high-risk HPV E5 proteins will provide insight into how the E5 proteins modulate HPV pathogenesis.

S14/P8

The interaction between the MDV-1 Neurovirulence factor PP14 and the host transcription regulator, CREB3

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Tropism for the central nervous system has been a consistent feature of highly virulent strains of Marek's Disease Virus (MDV). However, the mechanisms behind the MDV-induced neuropathology are still poorly understood. Reverse genetics studies and chicken challenge experiments revealed that the immediate-early viral protein, pp14, is linked to the neurovirulence phenotype of the virus. Further, yeast two hybrid screening identified the ER-resident transcription regulator CREB3 (cAMP Response Element-Binding protein) as an interacting partner of pp14. The interaction with pp14 indicated that CREB3 could be involved in the molecular mechanisms leading to the neurovirulence of MDV. The aim of my project is to characterise the interaction between pp14 and CREB3 using biochemical, cellular and structural approaches with a view to determining the role of these proteins in the neurovirulence of MDV in chickens. We identified that the N-terminal domain of CREB3 interacts with pp14. Furthermore, we engineered recombinant RB-1B viruses that express various CREB3 variants. Infection of chicken embryo fibroblasts (CEF) with these CREB3 expressing viruses revealed that the interaction of pp14 with CREB3 may alter during the course of infection. Furthermore, we aim to identify the region of pp14 that interacts with CREB3 and to characterize the interaction of pp14 with CREB3 on a structural level. Finally, we will assess the effect of the mutant viruses on MDV pathogenesis using *in vivo* challenge experiments.

S14/P9

Investigating the influence of agnoprotein on BK polyomavirus egress

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With an increase in the usage of immunomodulatory drugs, the incidence of disease associated with BK polyomavirus (BKPyV) is notably rising. The small dsDNA virus that asymptomatically infects >80% of the global population commonly leads to detrimental effects following renal and bone marrow transplantation, with graft rejection a potential prognosis. As no antiviral therapy is currently available, there is a growing need to identify potential drug targets to prevent disease caused by BKPyV. Alongside the early tumour antigens and late structural proteins, a small hydrophobic auxiliary protein termed agnoprotein is expressed. Of the known human polyomaviruses, only BKPyV and JC polyomavirus have been confirmed to express an agnoprotein. High sequence conservation between these agnoproteins, with consistencies in side chain polarity and structure, suggests many activities are likely to be preserved. Agnoproteins have been shown to interact with several host and viral proteins during the lifecycle of BKPyV; influencing critical processes in the production, maturation and egress of infectious virion progeny. There is evidence that a central α-helical domain is present within agnoproteins, which likely has importance to the functionality of the protein. Through side-directed mutagenesis, residues within the helix were targeted to create single point variants. Here it is reported that the substitution of phenylalanine to glutamic acid at residue 39 displays a phenotype comparable to an agnoprotein knock-out virus, whilst a more conservative alanine substitution maintains egress comparable to the wild type virus.

S14/P10

Coupling of a binding peptide to the hepatitis B core virus-like particle

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The hepatitis B core (HBc) virus-like particle (VLP) is emerging as an attractive vaccine platform for the presentation of epitopes. The HBc monomers dimerise and self-assemble into VLPs. The tandem

core HBc VLP technology is a significant advancement on the standard HBc technology in that the physical linking of the two individual monomeric subunits avoids the steric clashes associated with antigen presentation. Here we have presented antibody mimetics termed adhirons - peptides that specifically bind to selected targets with high affinity. As a proof of principle, we have incorporated an adhiron to the Small Ubiquitin-like Modifier (Sumo) into the major immunodominant region (MIR) of the cores, thus allowing the "decoration" of the particles by any Sumo-tagged protein. The VLPs were generated in E. coli and characterised using a wide variety of biophysical and biochemical methods.

S14/P11

Expression of small open reading frames during KSHV latent and lytic replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncovirus associated with multiple malignancies, including Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease. Like all Herpesviruses, KSHV is capable of latent and lytic infection of host cells. During latency the virus remains in a dormant state with limited gene expression. After reactivation, the virus enters the lytic phase and mediates subsequent production of infectious virions through an expression cascade of early, intermediate-early and late genes. Recent research provided a comprehensive annotation of the KSHV genome and identified the existence of several small open reading frames (smORFs) in the KSHV genome, which are capable of expressing peptides containing 100 amino acids or less. To date, very little is known about the role of these smORFs during the KSHV life cycles. In this study we overexpress KSHV smORFs to elucidate their function in KSHV latent and lytic replication. We confirm expression and localisation to specific, discrete subcellular compartments, suggesting a possible role during virus replication. Furthermore, using stable isotope labelling by amino acids in cell culture (SILAC)-based quantitative proteomics we identified translated smORFs at marked time points during the KSHV lytic expression cascade. Together we established an expression profile indicating a clear function for smORFs during KSHV infection and ongoing research is dissecting possible roles of these virally encoded smORFs.

S14/P12

A systematic investigation of the HCMV US12 gene family

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Human cytomegalovirus (HCMV) infects 40-100% of the population, causes complications in immuno-compromised or suppressed individuals such as those with HIV/AIDS and patients post-transplant, and is the leading infectious cause of birth defects. It encodes numerous genes that are potentially involved in modulating the host's immune defences and promoting virulence *in vivo*.

Two members of the US12 multi-gene family (US18 and US20) have recently been implicated in immune modulation of natural killer (NK) cell responses (Fielding *et al*., 2014). Since the US12 family have characteristics of a viral gene expansion, we hypothesised that they would all modulate immune recognition. NK assays and proteomic analysis revealed that 5 of the 10 US12 family members are NK evasion genes and that a number modulate NK/T cell ligands, cell adhesion molecules and cytokine receptors.

To complement this analysis, we are tagging each US12 family member using a C-terminal V5 epitope tag within the HCMV Merlin BAC. These recombinant viruses will allow us to determine US12 family expression kinetics, their interacting partners by stable isotope labelling in cell culture (SILAC)-immunoprecipitation, their subcellular localisation, glycosylation and presence in HCMV virions.

Our initial findings by immunofluorescence indicate that some US12 family proteins associate with the virion assembly compartment (vAC), whereas others have a distinct subcellular localisation. Additional experiments are ongoing to determine different localisation patterns using specific organelle markers, glycosylation and interactomes. These data will provide important information relevant to the function of the US12 family during HCMV infection and shed light on their mechanism of action.

S14/P13

Adhirons: A novel antibody-like technology for use in Virology

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Antibodies are frequently used in many techniques in virology from diagnostics to research applications. Production of antibodies is time consuming, expensive and may not produce a reagent sufficiently specific for the intended purpose with potential batch to batch variation. Alternative Artificial Binding Proteins (ABPs) are being developed to overcome these

limitations and to rapidly provide new tools for research, diagnostics and potentially therapeutic applications. We have screened a library of ABPs known as Adhirons which have a constant 91 amino acid scaffold and two variable 9 amino acid loops that are responsible for the recognition of target molecules. We have isolated Adhirons capable of specifically recognizing the UL49 encoded tegument proteins of two avian herpesviruses (Herpesvirus of Turkey and Duck Enteritis virus), these isolated Adhirons perform well in ELISA, In cell western and Immunofluorescence applications. Our isolated Adhirons are specific to their targets with biolayer interferometry indicating low nanomolar binding affinities for their cognate targets. Our work represents the first time this technology has been trialed for use in virology, where rapid generation of specific antibody-like reagents is of increasing importance to respond to new threats to animal and human health.

S14/P14

Development of a candidate reference material for adventitious virus detection by deep sequencing: report of an international collaborative study.

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Background Unbiased deep sequencing offers the potential for improved adventitious virus screening in vaccines and biotherapeutics. Successful implementation of such assays will require appropriate control materials to confirm assay performance and sensitivity.

Methods A common reference material containing 25 target viruses was produced and 16 laboratories were invited to process it using their preferred adventitious virus detection assay.

Results Fifteen laboratories returned results, obtained using a wide range of wet-lab and informatics methods. Six of 25 target viruses were detected by all laboratories, with the remaining viruses detected by 4 - 14 laboratories. Six non-target viruses were detected by three or more laboratories.

Conclusion The study demonstrated that a wide range of methods are currently used for adventitious virus detection screening in biological products by deep sequencing and that they can yield significantly different results. This underscores the need for common reference materials to ensure satisfactory assay performance and enable comparisons between laboratories.

S14/P15

The Role of DNA-PK in HSV-1 infection

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DNA protein kinase (DNA-PK) was recently identified as an innate immune sensor sensor of double-stranded DNA in the cytoplasm. Provisional results indicated that DNA-PK knock-out mice support an increased yield of herpes simplex virus 1 (HSV-1) at the site of viral infection, and it is thought that HSV-1 protein ICP0 induces the degradation of DNA-PK during infection. The HSV-1 genome is replicated in the nucleus, meaning that its genome may be exposed to nuclear sensors of double-stranded DNA. The primary aim of this study is to elucidate antiviral role of DNA-PK during HSV-1 infection, including its potential role as a nuclear sensor of viral DNA.

The use of primary cells is critical to the study of innate immune responses. For example it is known that primary murine embryonic fibroblasts (MEFs) lose responsiveness to cytoplasmic DNA stimulation after the first few passages. Hence primary murine skin fibroblasts (MSFs) are preferred to study these initial signalling events pathway. Here we present an initial characterisation of the innate response of wild type and DNA-pkcs null MSFs to HSV-1 infection and to cytoplasmic DNA stimulation.

S14/P16

Peroxynitrite is required for cytomegalovirus infection in vitro and in vivo

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How cytomegalovirus exploits host cellular pathways during replication requires a better understanding. Herein we report a critical role for peroxynitrite, a reactive nitrogen species generated from a reaction between nitric oxide and superoxide, in supporting replication of cytomegalovirus infection. The peroxynitrite decomposition catalyst FeTPPS dramatically reduced murine cytomegalovirus (MCMV) replication in fibroblasts and macrophages in vitro, and inhibited virus replication and associated weight loss in vivo. Moreover, treatment of THP1 myeloid cells antagonized human cytomegalovirus (HCMV) replication, demonstrating a cross-species effect. The NADPH oxidase complex is a significant source of superoxide, and we hypothesised that this complex may promote cytomegalovirus replication. In accordance, mice deficient in Essential for Reactive Oxygen Specifies (EROS) were partially resistant to MCMV replication. Thus these data demonstrate the importance of the superoxide-peroxynitrite pathway in cytomegalovirus replication.

S14/P17

Intracellular chloride channels are involved in Merkel cell polyomavirus ST-induced cell motility

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Merkel cell polyomavirus (MCPyV) is the causative agent of Merkel cell carcinoma (MCC), a highly aggressive and metastatic cancer of neuroendocrine origin. We have previously shown that MCPyV small tumour antigen (ST) expression induces cell motility via microtubule dissociation and the formation of motile structures, such as filopodia. Here we demonstrate that MCPyV ST-expressing cells have increased chloride ion efflux and that intracellular chloride channels contribute to MCPyV ST-induced cell motility and filopodia formation. Treatment of MCPyV ST-expressing cells with broad-spectrum chloride channel inhibitors abrogates MCPyV ST-induced cell motility and reduces MCPyV ST-induced filopodia formation. In addition, siRNA-mediated depletion of two specific chloride channels, CLIC1 and CLIC4, known to be involved in multiple types of cancer, also reduce MCPyV ST-induced cell motility. These data show that the activity of intracellular chloride channels contribute to MCPyV ST-induced cell motility and suggest a new avenue of targeted drug therapy to inhibit the highly metastatic nature of MCC.

S14/P18

CD4 and CD8 T cell response to human cytomegalovirus proteins expressed by latently infected cells

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During HCMV latency, the viral transcription programme is very limited compared to the lytic cycle. We previously showed that two viral gene products expressed during latency, UL138 and LUNA, are recognised predominantly by $CD4^+$ T cells, which secrete IFNγ, show MHC class II-restricted cytotoxicity and can recognise latently infected cells. In contrast, a small proportion also secreted the immunosuppressive cytokines IL-10 and transforming growth factor β (TGF-β).

Little is known about the host immune response to other viral proteins expressed during latency; including US28, UL111a, and UL144. We now show that these latency-associated viral gene products are also T cell targets.

In a large donor cohort of 50 individuals aged between 23–74 years old, we measured T cell responses using a dual IFNγ/IL-10 FluoroSpot assay. Similar to UL138 and LUNA, we observed recognition of US28, UL111a, and UL144 ORFs by $CD4^+$ T cells; these T cells secreted IFNγ and a sub population secreted IL-10. We also observed CD^8+ IFNγ secreting T cell responses to these latent antigens.

T cell responses to UL111a and LUNA were generally lower frequency than responses to US28 and UL144. Intriguingly, there was a significant bias of UL138 specific T cell responses detectable in young donors compared to middle/old age donors.

Our results suggest that, although the major response to latency-associated antigens comprise $CD4^+$ T cells, some viral proteins expressed during latency also elicit $CD8^+$ T cell responses and future work will attempt to determine why this recognition does not result in a clearance of latency.

S14/P19

An automated framework to aid the taxonomic classification of DNA virusesAn automated framework to aid taxonomicclassification of DNA viruses

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Viruses are classified formally into orders, families, genera and species. Various criteria may be used to make taxonomic assignments, with phylogenetic relationships based on sequence data being prominent among them. Due to advances in sequencing technology and falling costs, the amount of sequence data deposited into public repositories is increasing daily. This poses a major challenge to update, integrate and maintain phylogenies of viral families. We have developed a bioinformatics framework that automatically selects new candidate virus sequences from GenBank, generates multiple sequence alignments, calculates a maximum likelihood phylogeny and integrates the sequences into the existing phylogenetic trees. Initially, all known protein sequences available in GenBank are downloaded, avoiding filtering based on GenBank annotations. BLAST is then used to compare these sequences with a seed set consisting of a curated set of sequences spanning the known diversity of the family. Significant matches are extracted based on the e-value and a pre-defined length parameter, followed by multiple sequence alignment and RAxML maximum likelihood tree generation. These data can be updated when new sequences are available in GenBank and all versions of the tree and alignments are retained for future reference using version control. The latest version of the tree is submitted to an interactive online tree visualisation tool, which combines the tree with pairwise distance data and enables user to employ filtering based on defined cut-off values. The pipeline is currently set up with *Herpesviriadae* and *Parvoviridae* families but is flexible and can be adapted for any virus family.

S14/P20

Structure of the Intranucleus Herpes Virus Capsid from Intact Vitrified Frozen Cell Sections by Cryo Electron Tomography

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Cryo-EM imaging and reconstruction of frozen vitrified samples has become the method of choice for analysing the structure of medium to large complexes such as viruses and macromolecular assemblies. Until recently, most such analyses have been confined to purified samples. However, there has been increasing interest in extending studies into cells where the majority of biologically interesting structures and processes are located. Limited penetration by the illuminating electrons prevents imaging of specimens thicker than ~500 Å, restricting this form of analysis to thinner samples, such as intact bacterial cells and peripheral regions of some eukaryotic cells. To overcome this limitation, sectioning methods have been employed including cryo-sectioning of (CEMOVIS) and focussed ion beam milling of (cryo-FIB) vitrified specimens. However, these methods are technically challenging, subject to artefacts (CEMOVIS), or involve specialised equipment of limited availability (cryo-FIB). Here we describe the application of the well-established Tokuyasu sectioning method for preparing material for imaging as vitrified sections by cryo-EM tomography. In this procedure, fixed samples are infiltrated with sucrose, which acts as a cryoprotectant, allowing easy sectioning on a standard cryo-microtome. The cut sections are thawed and washed to remove the sucrose, before being vitrified and imaged in the cryo-EM. Using this approach, we have carried out sub-tomogram averaging to generate low resolution icosahedral structures of intranuclear herpesvirus capsids. To our knowledge, this represents the first direct determination of virus structure from within the nucleus of a cell using cryo-EM techniques.

S14/P21

A Bioinformatics Approach to Characterise Phaeovirus Infection

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All viruses infect their hosts using one of two strategies. Acute strategist viruses are the better described, being typically associated with a disease phenotype and novel emerging strains, whereas Persistent strategist viruses are associated with low levels of replication and mutation. Phaeoviruses are the only known giant algal virus to employ a persistent infection strategy. They integrate into the genomes of Ectocarpoids, disrupting their reproductive efficiency upon expression. Using Next Generation Sequencing techniques we isolated the viral genome and transcriptome of the Phaeovirus Feldmannia irregularis for analysis. We describe 4 novel variants of the single copy core genes Major Caspid Protein, DNA polymerase and a viral superfamily III Helicase which between them contained 13 amino acid polymorphisms. It is proposed that the loss of a DNA proofreading mechanism, coupled with high levels of genetic variation observed in genes involved in DNA replication are the main contributors to the number of mutations observed. Our findings support the theory that Feldmannia irregularis virus displays a unique evolutionary strategy, retaining a persistent infection strategy while displaying high levels of mutation more typically associated with acute strategist viruses.

S14/P22

Giant host, giant virus: genetics and microscopy of kelp phaeoviruses

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The giant DNA viruses (genus Phaeovirus) of brown algae (class Phaeophyceae) employ a unique latent infection strategy with replication restricted to algal reproductive structures, and are currently only known in ectocarpoid seaweeds (Ectocarpales). Phaeoviral infection of kelp (Laminariales), a group closely related to ectocarpoids, has been found for the first time. A phaeoviral gene encoding major capsid protein (MCP) was found in 64.7% of kelp sporophytes (macroscopic life cycle stage). Phylogenetic trees showed the kelp virus MCP sequences to form a tentative group within Phaeovirus, referred to as Laminariales Virus. We suggest more variable genes would reveal a distinct sub-group of phaeoviruses infecting kelp hosts. Confocal microscopy of MCP-positive kelp gametophytes (microscopic life cycle stage) showed abnormal reproductive structures filled with opaque material resembling the masses of phaeoviral particles seen in ectocarpoids. We propose the known Phaeovirus host range to include kelp and the first insights into the prevalence, phylogeny, and infection strategy of kelp phaeoviruses, with future questions concerning the ecosystems and industries founded on kelp.

S14/P23

Epidemiology of canine adenovirus type 1 in red foxes (*Vulpes vulpes*) in the UK.

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Canine adenovirus type 1 (CAV-1) causes infectious canine hepatitis (ICH) in domestic dogs and wild canids, including red foxes (*Vulpes vulpes*). Lesions in fatal ICH include severe hepatic necrosis and disseminated intravascular coagulation caused by

viral replication in hepatocytes and vascular endothelial cells. A molecular and serological survey was conducted on samples from red foxes in the UK, to investigate whether this species is a wildlife reservoir of CAV-1. The findings indicate that in some regions around 30% of red foxes, without evidence of disease due to ICH, have detectable CAV-1 sequences, that viral copy numbers differ amongst tissues and that a number of infected animals also shed CAV-1 in urine. These animals are therefore a potential source of infection for conspecifics and domestic dogs. Post-mortem examination of red foxes revealed that none of the infected animals had gross or histological evidence of ICH. It is hypothesised that a mechanism of host evasion/viral persistence exists in CAV-1 infections, allowing the virus to persist in host tissues without evidence of disease.

S14/P24

Metagenomics approach for the detection and characterisation of a selection of DNA viruses associated with causing encephalitis

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Acute encephalitis is an inflammation of the brain parenchyma. It is mostly caused by viral infections and autoimmune disorders, although in more than 37% of the patients the specific cause remains undetermined. Mortality rates range between 7-18% and severe disability has been reported in up to 56% of survivors. Metagenomics, the direct analysis of genetic material contained within a sample without prior knowledge about its makeup can be used for the discovery of novel viruses and to detect and characterise different known viruses in a single assay.

We set out to establish a metagenomics approach suitable for the diagnosis of viral encephalitis. A sample consisting of a mixture of 11 encephalitis-associated DNA viruses namely; HSV-1, HSV-2, VZV, EBV, CMV, HAdV, HHV-6A, HHV-6B, BKV, JCV and B19V, with viral loads between 10-105 copies/mL, was obtained from NIBSC. Viral nucleic acid was manually extracted and used for DNA library preparation followed by massive parallel sequencing on a MiSeq System (Illumina). A bioinformatics pipeline that assigns taxonomic labels to short DNA reads was used for viral identification followed by reference mapping. Complete genome consensus sequences were generated for HAdV, BKV and JCV whereas partial genomes, ranging in coverage from 60-90% were obtained for the other 8 viruses. We blindly tested 8 CSF samples from patients diagnosed with viral encephalitis using this approach. In all the cases diagnosis results by conventional methods of EBV, HSV2, HHV6A were confirmed by metagenomics. This showed that the technique could be suitable for diagnosis of viral encephalitis.

S14/P25

Viral communities of the deep-sea anoxic brines of the Red Sea

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The brine-filled deeps of the Red Sea are among the most extreme and inaccessible environments on Earth. They combine multiple environmental extreme conditions (e.g. increased salinity, temperature and heavy metal concentration, as well as anoxia). They remain largely underexplored despite a recent surge in scientific exploration, which uncovered an unexpectedly rich biological diversity and identified several novel extremophilic groups.

The current study surveys four metagenomic datasets obtained from the brine-seawater interfaces of different deeps as a way of exploring the viral communities present in these environments.

Our analyses show that the particle-attached viral communities vary from sample to sample but are generally quite diverse and dominated by *Caudovirales*. The most divergent profile was obtained from the unusual "hot brine" of the Atlantis II deep, which had a much higher proportion of unclassified viruses and pointed to a higher potential for novelty. Another noteworthy highlight was the demonstration of clear stratification in the viral community across the brine-seawater interface. This mirrors changes in microbial community and in the physical-chemical conditions when moving from seawater to the brine.

Our findings provide the very first insights into the virology of these unusual ecosystems in the Red Sea and are the first step for future sampling efforts and studies.

S14/P26

STING affecting Nucleo-Cytoplasmic Shuttling of a Novel Innate Immunity Regulator

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STING is a transmembrane sensor that initiates innate immune responses (IIR) by recognising pathogen DNA in the ER. Along with DNA viruses it also functions against RNA viruses, but the mechanism is unclear. Here we find a separate nuclear envelope role for STING, directing the nucleo-cytoplasmic distribution of RNA-binding proteins that we here show contribute to IIR. These were among 17 nuclear envelope-specific STING binding partners predicted to indirectly affect the IRF3/7 transcription factors that activate IIR genes. These RNA binding proteins normally redistribute/shuttle between the nucleus and cytoplasm during IIR and STING knockdown blocked this nucleo-cytoplasmic shuttling. Interestingly, the directionality was affected with Herpes Simplex Virus (HSV-1) infection.

Transport through the nuclear pore central channel is often targeted by pathogens; thus these data suggest a model whereby the membrane embedded STING provides a backup system for activating IIR by redistributing signaling proteins via the nuclear pore.

S15/P1

Results of molecular proficiency for measles testing in UK

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Molecular testing for measles has been in place at Virus Reference Department, Colindale, since 1994, and is an invaluable tool for diagnosis of measles in the first three days of illness. Following the large outbreak of measles in England in 2012-2013, a decision was made to roll-out measles molecular testing across all PHE regional laboratories (PHLs) to offer localised testing for diagnosis of acute measles cases.

To monitor performance of PHLs in detecting measles, a Measles Molecular Proficiency Testing Program was introduced. The 1st panel was distributed to the eight PHLs in March 2014, and the 2nd to all eight PHLs and to four laboratories in the devolved administrations in October 2015.

The proficiency panels comprised 18-20 samples including negatives and positives with various viral loads, and human cellular content. The positives were formed from 4 or 5 different measles genotypes, and rendered non-infectious prior to distribution.

The results from panel 1 demonstrated the capability of all eight PHLs to perform molecular detection for measles; the accuracy of detecting different viral loads was excellent. There were no significant issues or critical errors. Following a recommendation from WHO, the second panel also included the detection of human cellular content to determine whether the sample was suitable for testing. The results of the 2nd proficiency panel are still being analysed and will be presented. Continuing to monitor the performance of the participating laboratories is critical for maintenance and enhancement of the current measles surveillance system, especially in an era of measles elimination.

S15/P2

Production and characterisation of monoclonal antibodies for human papillomavirus serology, to be used as 1st WHO International Standards

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Human Papilloma virus (HPV) vaccine programmes have been introduced worldwide to reduce the incidence of HPV-associated cancers. Three vaccines have so far been approved, based on virus like particles (VLPs). These are a bivalent vaccine comprising high risk HPV type 16 and 18, which together are responsible for more than 70% of the HPV-associated cancers. A quadravalent vaccine which also includes types 6 and 11 which are associated with genital warts and the recently licensed nonavalent vaccine also includes types 31, 33, 45, 52 and 58. The quality of these VLPs is important for their immunogenicity, and the WHO Expert Committee on Biological Standardization (ECBS) has endorsed the production of a panel of monoclonal antibodies (mAbs) specific to intact VLPs, to be freely available, for use in VLP quality control and serological assays for monitoring vaccine efficacy and epidemiology. These mAbs have been characterised using a number of techniques to identify homologous and cross reactive binding and neutralisation activity, affinity and potential epitope mapping. Currently mAbs to types 16 and 18 are being examined, but future work will include the production of mAbs to the HPV types comprising the nonavalent vaccine, alongside a novel pan HPV mAb to a linear peptide from HPV L1 protein.

S15/P3

Testing for Hepatitis C viraemia following a new diagnosis of positive serology in primary and secondary care

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There has been a recent rapid expansion in the number of classes of antiviral drug available for the treatment of Hepatitis C Virus (HCV), leading to the possibility of treatment and cure in many patients for whom older regimens were unsuitable or unsafe. The aim of this audit was to ascertain how many patients with a new diagnosis of positive HCV serology are followed up by polymerase chain reaction (PCR) testing to detect viraemia. **Methods:** Consistent with published guidelines, the auditable standard was 100% PCR testing. We retrospectively searched for all adult patients in the local catchment area who were tested for HCV serology in primary or secondary care between 1st November 2011 and 31st October 2014. We excluded deceased organ donors. **Results**: We found 786 unique patient records of first-positive HCV serology results; 73% were male and the median age was 45 years. 48% of tests had arisen from a location other than the hepatology/transplant ward or clinics, with 22% from GPs, 3.3% from prisons and 3.1% from Genitourinary Medicine (GUM) clinics. 80% of new HCV serology results were followed by PCR testing. However, of those who were not tested by PCR, 44% came from GPs, 11% from prisons, and 6% from GUM clinics. **Conclusion:** These data suggest that a significant number of adults, who may now be considered eligible for new treatments, have not been tested for HCV viraemia and may benefit from future testing.

S15/P4

Influenza hide and seek - detection of stalk directed antibodies

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The influenza virus is dynamic, drifting and shifting its way around our immune defences in order to propagate itself, resulting in widespread morbidity and mortality, and a very high economic burden.

The quest to find a 'universal' vaccine directed against the more conserved regions of our immune systems primary viral target: haemagglutinin (HA) - is ongoing. However, accurately measuring the immunogenicity of such vaccines is lagging behind.

There are few techniques to successfully identify HA2 stalk targeting and neutralising antibodies from candidates vaccinated with promising universal vaccines. Microneutralisation assays based on lentiviruses pseudotyped with chimeric haemagglutinins are one solution.

A panel of chimeras have been produced with H1 stalks and heterologous globular head domains to which the human population is largely naïve. This results in a surrogate serological virus antigen that can be used to distinguish between general neutralising antibodies and those that are specific to the stalk.

To date we have produced three different pseudotypes bearing chimeric HAs from H1 strains of human importance - 1918pdm, 2009pdm and a 2006 vaccine strain. This study focuses on the exploitation of these novel chimeric HA pseudotypes for the delineation of humoral antibody responses that may be relevant for future vaccine development.

Subtype specific antiserum as well as stalk-neutralising monoclonal antibodies were used to test this system, our chimeras were poorly neutralised by antiserum against the subtype of the stalk origin, while still being knocked down by stalk-directed monoclonal antibodies. Data pertaining to the validation of this system will be discussed.

S16/P1

Vaginotypes of the Vaginal Microbiome of Pregnant Women

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Background: The knowledge of the community structure of the vaginal microbiome of pregnant women is limited. Few comparisons have been made between the microbiota of healthy gravidae women and those who experienced complications during pregnancy; in particular women giving birth at term or preterm, This investigation addresses these questions.

Methods: Upper vagina swabs were obtained from 228 women of Caucasian, Asian, Indian, Middle East, and Pacific Island racial backgrounds during the third trimester of pregnancy. Participating women were administered a questionnaire including demographic data and summarising the history of the pregnancy; 135 women of five ethnic backgrounds had no complications during the pregnancy. The identity of the taxa present was determined by ultrafast sequencing of the V1V3 regions of the 16S rDNA gene of DNA extracted from the swabs. The sequence data were analysed with MOTHUR and statistical analyses were performed employing various bioinformatics tools.

Results and Discussion: The relative abundance of some phyla was different in pregnant and non-pregnant (from other studies) women. Multidimensional scaling analyses showed the bacterial populations of women without complications to cluster in four vaginotypes driven by four Lactobacillus species. This organisation was partially dependent on the racial background of the woman and was altered by complications during pregnancy. A CLUSTER analysis showed a fine structure in these vaginotypes created by taxa belonging to the genera Escherichia, Atopobium and Prevotella. The participants were stratified into two groups: with or without complications during pregnancy. PERMANOVA analyses yielded significant differences between the microbiomes of both groups.

S16/P2

CamOptimus: A self-contained, user-friendly multi-parameter optimisation platform for non-specialist experimental biologists

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Biological problems are usually complex due to their multi-parametric nature and to the fact that these parameters are often interdependent. A commonly employed approach in attacking such problems relies on the use of background knowledge, or informed guesswork, to prioritise the investigation of these parameters. For novel systems, there may be insufficient background knowledge to enable successful prioritisation. Moreover, identifying and testing the effect of individual parameters is often an ineffective strategy because it ignores the interactive effects of mutually dependent parameters. We have developed a hybrid approach adopting the most desirable features of the Design of Experiments (DoE) and Genetic Algorithm methodologies to solve this multi-parametric experimental design problem. We present here a simple-to-use and freely available software package and graphical user interface (GUI) to empower a wider audience of experimental biologists to employ GA in solving their optimisation problems. This hybrid approach allows experimental biologists to: (i) collect extensive data over the complete solution space, (ii) describe the solution space and how the output is affected by the dependent parameters under investigation, and (iii) identify the exact optimal solution in the global optimum sub-space refined over the generations. We believe this new tool to be an attractive alternative to the commercially available DoE software for experimentalists in both academia and SMEs.

S16/P3

NMR Metabolic profiling of a yeast ALS proteinopathy model overexpressing wild type and mutant optineurin

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Optineurin (OPTN) is a cytosolic protein that was originally associated with glaucoma, and whose known functions include a role in autophagy. Mutations in the OPTN gene have been found to be linked to amyotrophic lateral sclerosis (ALS) in humans. As a first step towards understanding the effect of OPTN overexpression at the metabolic level, a yeast model system has been constructed that overexpresses 2 forms of OPTN, C-terminally tagged with YFP: wild-type and the E478G mutation linked to ALS and glaucoma in humans. We found that OPTN overexpression was toxic for yeast and leads to slower growth. Using NMR, the metabolic profiles of yeast overexpressing wild-type and E478G OPTN have been obtained in order to identify, by use of multivariate statistical analysis, the key metabolites which change in concentration in cells expressing the human protein. Initial results indicate that NAD and branched-chain amino acids vary between untransformed yeast strains and those overexpressing human OPTN. Metabolic profiles of both hydrophilic and hydrophobic metabolites have been analysed, since sphingolipids have been identified as being important in glaucoma patients. Identification of the metabolic changes accompanying OPTN overexpression may help to shed light on the mechanism of OPTN toxicity in ALS patients.

S17/P1

Alkaline protease as detergents and solvents compatible nanobiocatalyst via immobilization onto functionalized rattle-type magnetic core@mesoporous shell silica nanoparticles

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Alkaline protease from alkaliphilic Bacillus sp. NPST-AK15 was immobilized onto functionalized and non-functionalized rattle-type magnetic core@mesoporous shell silica (RT-MCMSS) nanoparticles by physical adsorption and covalent attachment. However, the covalent attachment approach was superior for NPST-AK15 protease immobilization onto the activated RT-MCMSS-NH_2 nanoparticles and was used for further studies. In comparison to free protease, the immobilized enzyme exhibited a shift in the optimal temperature and pH from 60 °C to 65 °C and pH 10.5 to 11.0, respectively. While free protease was completely inactivated after treatment for 1 h at 60 °C, the immobilized enzyme maintained 66.5% of its initial activity at similar conditions. The immobilized protease showed higher *Vmax*, *k*cat and *k*cat/*Km*, than the soluble enzyme by about 1.3-, 1.3-, and 1.6-fold, respectively. In addition, the results revealed significant improvement of NPST-AK15 protease stability in variety of organic solvents, surfactants, and commercial laundry detergents, upon immobilization onto activated RT-MCMSS-NH2 nanoparticles. Furthermore, the immobilized protease maintained significant catalytic efficiency for ten consecutive reaction cycles, and was separated easily from the reaction mixture using an external magnetic field. To the best of our knowledge this is the first report about protease immobilization onto rattle-type magnetic core@mesoporous shell silica nanoparticles. The results clearly suggest that the developed immobilized enzyme system is a promising nanobiocatalyst for various bioprocess and protease applications.

S17/P2

Characterization of Candida famata Isolated from Poultry Feces for Possible Probiotic Applications

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Abstract
We studied here, the yeast content of poultry feces collected randomly from a French farm located in the north of the country. Thus, 81 yeast colonies were isolated and clustered into 22 distinct groups using the rep-PCR method. A single colony was taken from each group and identified using biochemical (ID 32C system), and molecular (sequencing of the D1/D2 domain of 26S rDNA and ITS1-5.8-ITS2 rDNA region) methods. Both methods led to the identification of *Candida famata* species. One isolate of *Candida famata* strains, named strain Y5, was studied for its cytotoxicity, adhesion, surface properties, hemolytic activity, survival in simulated gastric and intestine environments. Overall, the data obtained advocate the probiotic potential of this isolate.

Keywords: Yeasts, Poultry feces, *Candida famata*, rep-PCR, probiotics

S17/P3

Effect of carrier-based Rhizobium leguminosarum inoculants on the soil physicochemical characteristics, nodulation and growth of soybean

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Inoculation of legumes with beneficial rhizobacteria is a desirable practice to improve plant growth and productivity. A field experiment to evaluate the effect of carrier-based *Rhizobium leguminosarum* inoculants on the soil physicochemical characteristics, nodulation and growth of soybean was carried out. Sawdust and cassava peels were the carriers used in this study. *Rhizobium leguminosarum* used was isolated from soil. The physicochemical characteristics of the carriers were determined and total bacteria and fungi counted. The sawdust and cassava peel carriers had water holding capacities of 4.2 and 6.0 ml/g, pH of 6.60 and 6.70, respectively. The physicochemical characteristics of the soil at the experimental site before the field experiment, showed a pH of 6.65 and total Nitrogen of 1.57%.The physicochemical characteristics of the soil after 40 days of planting with cassava peels-based *Rhizobium leguminosarum* showed a pH of 6.68 and total Nitrogen of 2.37% while the sawdust-based *Rhizobium leguminosarum* showed a pH of 6.66 and total Nitrogen of 2.06% with control having a total Nitrogen of 1.51%.The shoot height was significantly different from the control at $p \leq 0.05$, though other growth parameters

were not statistically significant from the control. Nonetheless, there was marked increase over the control in nodulation and other growth parameters with the highest found in soybean plant inoculated with cassava-based *Rhizobium leguminosarum*. This study therefore showed that since the carrier-based *Rhizobium leguminosarum* inoculants increased the physicochemical characteristics of the soil, nodulation and soybean growth, such inoculant is an effective alternative to chemical fertilizers in enhancing plant growth.

S17/P4

A new subclas of Beta-Carbonic Anhydrases in Clostridium autoethanogenum and Clostridium ljungdahlii?

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Carbonic anhydrases (CAs, EC 4.2.1.1)) are enzymes that catalyse the reversible conversion of carbon dioxide and water to bicarbonate and protons. Both carbon dioxide and bicarbonate ions are important substrates in metabolism of most organisms and CAs have been studied in all mayor branches of the tree of life.

CA activity in the closely related *Clostridium autoethanogenum* and *Clostridium ljungdahlii* has not been previously described. These acetogenic autotrophs are used in industry to fix carbon monoxide and carbon dioxide into organic matter and valuable products. For a complete understanding of the metabolism of these cells it is necessary to understand the role CAs play.

We identified two putative carbonic anhydrase genes in the genomes of *C. autoethanogenum* and *C. ljungdahlii.* One codes for a β- carbonic anhydrase (β-CA) with little similarity to other known β- CAs and is a member of a novel sub-class of β-CAs. The other is a γ-carbonic anhydrase (γ-CA) with clear homology to other proteins in the γ-CA protein family.

Both genes were heterologous expressed in *Escherichia coli* and purified using Immobilized metal ion affinity chromatography. To determine CA activity of these purified enzymes a CA assay was developed using a 96-well plate reader with automated injection.

The β-CA of *C. autoethanogenum* shows CA activity while the γ-CA seems not to be active. ClosTron insertional mutants were generated of both CA genes of *C. autoethanogenum*. Further characterisation of the enzyme activity, gene overexpression and gene knock-out mutant strains is ongoing.

S17/P5

Water sources as reservoirs of *Vibrio cholerae* O1 and non-O1 strains in Bepanda, Douala (Cameroon): relationship between isolation and physico-chemical factors

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Abstract

In Douala, the major cholera endemic area of Cameroon, most outbreaks start from Bepanda, an overcrowded locality with poor hygiene and sanitary conditions. We investigated water sources in Bepanda as reservoirs of *Vibrio cholerae*, the cholera pathogen, determined its antibiotic susceptibility and some physico-chemical characteristics that could maintain the endemicity of this organism in Bepanda.

Three hundred and eighteen water samples collected from 45 wells, 8 taps and 1 stream from February to July 2009 were analyzed for *V. cholerae* using standard methods. Isolates were characterized morphologically, biochemically and serologically. The disc diffusion technique was employed to investigate antibiotic susceptibility.The seasonal variation of the organism prevalence were analyzed, together with its relationship to water temperature, pH and salinity.

Eighty-seven (27.4%) samples were positive for *V. cholerae.* Isolation was highest from wells. The organism was isolated in the rainy and dry seasons but the frequency of isolation was significantly higher in the rainy season. Of the 96 confirmed V. *cholerae* isolates, 32 (33.3%) belonged to serogroup O1 and 64 (66.6%) were serogroup non-O1/non-O139. Isolates from taps were non-O1/non-O139 strains. Salinity had a significant positive correlation with isolation in both seasons. Regression analysis revealed that as pH increased, odds of isolation of *V. cholerae* also increased. All isolates were sensitive to ciprofloxacin and ofloxacin.

Cholera will continue to be a health threat in Douala, if intervention measures to prevent outbreaks are not implemented. Continuous monitoring of water sources in Bepanda and other cholera high-risk area in Cameroon is therefore necessary.

S17/P5

FABRICATION OF A CONTINUOUS FLOW SYSTEM FOR BIOFILM STUDIES

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Modern and current models such as flow cell technology which enhances a non-destructive growth and inspection of the sessile microbial communities revealed a great understanding of biofilms. A continuous flow system was designed to evaluate possibility of biofilm formation by *Escherichia coli* DH5α on the stainless steel (type 304) under continuous nutrient supply. The result of the colony forming unit (CFU) count shows that bacterial attachment and subsequent biofilm formation on stainless steel coupons with average surface roughness of 1.5 ± 1.8 µm and 2.0 ± 0.09 µm were both significantly higher ($p \leq 0.05$) than those of the stainless steel coupon with lower surface roughness of 0.38 ± 1.5 µm. These observations support the hypothesis that surface profile is one of the factors that influence biofilm formation on stainless steel surfaces. The SEM and FESEM micrographs of the stainless steel coupons also revealed the attached *Escherichia coli* DH5α biofilm and dehydrated extracellular polymeric substance on the stainless steel surfaces. Thus the fabricated flow system represented a very useful tool to study biofilm formation under continuous nutrient supply.

S17/P6

Examining The Role of Soil pH on the Composition and Abundance of Nitrite Oxidising Bacteria

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Abstract
Nitrification, the microbial oxidation of ammonia to nitrate (NO3-) via nitrite (NO2-) is a vital process in the biogeochemical nitrogen cycle and is performed by two distinct functional groups; ammonia oxidisers (comprised of ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA)) and nitrite oxidising bacteria. Published studies revealed that soil pH is a major driver for determining the distribution and abundance of AOB and AOA. To determine whether distinct populations of nitrite oxidising bacteria within the lineages of Nitrospira and Nitrobacter are adapted to a particular range of pH as observed in ammonia oxidising organisms, the community structure of Nitrospira-like and Nitrobacter-like NOB were examined across a pH gradient (4.5 - 7.5) by amplifying nitrite oxidoreductase (nxrA) and 16S rRNA genes followed by denaturing gradient gel electrophoresis (DGGE). The community structure of both Nitrospira and Nitrobacter changed with soil pH, with distinct populations observed in acidic and neutral soils. The abundance of Nitrospira-like 16S rRNA and Nitrobacter-like nxrA gene copies contrasted across the pH gradient. Nitrobacter-like nxrA gene abundance decreased with increasing soil pH, whereas Nitrospira-like 16S rRNA gene abundance increased with increasing pH. Findings indicated that abundance and distributions of soil NOB is influence by soil pH.

Keywords: Nitrospira, Nitrobacter, Nitrite-oxidizing bacteria, Nitrification, pH, and Soil.

S17/P7

In silico characterization of PAS domains in selected Actinobacteria.

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Functional domains are semi-autonomous parts of proteins having their own structure and function. The Per-Arnt-Sim (PAS) domain exhibit in all living organisms and play a pivotal role in signal transduction pathway. They make associations with different co-domains to modulate their function. In the present study, we analyzed PAS domains found in some selected Actinobacteria inhabiting in different niche. Identification of domains and their characterization was solely based on *in silico* approach. Presence of the PAS proteins under the COG T (signal transduction) category implies their role in signal transduction. PAS proteins were found to be moderately expressed and structurally conserved. They showed a strong bias towards the lagging strand which indicates their involvement in adaptive evolution. Analysis of the PAS domains tertiary structure revealed a correlation between the structure and their biological network. From structure-based phylogenetic study it was observed that PAS domains, with similar co-domains, grouped together in a cluster. Thus, the tertiary structure of PAS domains might influence the association of co-domains and corresponding biological networks or vice-versa, facilitating proper niche adaptation.

S17/P9

Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from gate passes used for vehicular admittance and automated teller machines (ATMs) located within the premises of a university in southeastern Nigeria

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Vehicular gate passes and automated teller machines (ATMs) are the major, if not the only likely, items shared among campus visitors, students and staff. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) on vehicular gate passes and ATMs of Nnamdi Azikiwe University Awka, Anambra State, Nigeria over a 24-week period was investigated. *S. aureus* was isolated from 30% (108/360) of plastic gate passes and 44.9% of ATMs (151/336). *mecA* was identified in *S. aureus* from both gate passes (14.8%; 16 out of 108 presumptive positives) and ATMs (21.9%; 33 out of 151 presumptive positives). Multidrug resistance among isolated MRSA from both sources was recorded (ampicillin 0.5 µg/ml; ceftriaxone 64 µg/ml; ciprofloxacin 4 µg/ml; erythromycin 8 µg/ml; gentamicin 16 µg/ml; oxacillin 4 µg/ml; penicillin G 0.25 µg/ml; rifampin 4 µg/ml; streptomycin 1,000 µg/ml; tetracycline 16 µg/ml; and vancomycin 16 µg/ml). Implicated MRSA in samples present severe health-risk potentiality together with extensive adverse antibiotic resistance.

S17/P10

Antimicrobial actvity of *Nauclea lotifolia* (African peach) crude extracts against some pathogenic microorganism.

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The phytochemical screening and antimicrobial activity of *Nauclea lotifolia* fruit, leaf and stem-bark extracts at various concentration of (20.0,10.0, 5.0,and 2.5 mg/ml) were evaluated against some pathogenic microorganisms such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Aspergillus niger* and *Candida albicans*. The antimicrobial activity was assayed using agar well diffusion method. The result obtained show appreciable inhibitory effort of acetone, aqueous and methanolic extracts of *Nauclea lotifolia*. However , result obtained was less active compared to that of the control antibiotic (Ciprofloxacillin). The minimum inhibitory concentration (MIC) was determined using serial doubling dilution method and ranged from 5.0-10.0mg/ml, while the minimum bactericidalconcentration (MBC) was determined by plating various dilution of extracts without turbidity and the result ranged from 5.0-7.5mg/ml. The phytochemical screening revealed the presence of alkaloid, anthraquinones, flavonoids, resin, steroid and saponin. The activity of the plant extract therefore justify their utilization in the treatment of various ailments associated with the test organism.

S17/P12

Investigation of neutral lipid production by the yeast Debaryomyces hansenii

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Three yeast strains (*Debaromyces hansenii, Cryptococcus curvatus*, and *Yarowia lipolytica*) were screened for their ability to accumulate neutral lipid under osmotic stress conditions. Increasing NaCl concentration up to 0.8 M was accompanied by increases in intracellular solutes, and the highest content was in *C. curvatus* followed by *D. hansenii* which about 3.30 and 2.99 µg/µl respectively. From NMR analysis, it was found that glycerol was the main osmolyte accumulated in response to NaCl, and the maximum content was at 0.8 M NaCl especially for *D. hansenii*, and *C. curvatus*. After optimising the conditions for Nile red technique which was used to measure neutral lipid inside the cells, the results showed that the fluorescence intensity of Nile red in the logarithmic phase was higher than those in stationary phase, and the maximum intensity was found for *D. hansenii* grown at 0.8 M NaCl. Therefore, D. hansenii was chosen for further investigation to optimize cultivation conditions for lipid production. Two different media (minimal and YM medium) were used to quantify the influence of NaCl, carbon and nitrogen concentrations on neutral lipid yield. The accumulation of glycerol was significantly higher (LSD ≤ 0.01) with increasing salinity, especially in 1.6 M NaCl minimal culture (1360 µg glycerol/ mg biomass. Along with glycerol, neutral lipid production was found to be significantly higher in minimal medium than enriched medium. The highest lipid content was for 0 M NaCl grown cells which accumulated about 0.152 mg neutral lipid / mg biomass i.e. about 15% (w/w).

S17/P13

Investigating behavioural differences amongst high-performing *Pseudomonas* spp. surfactants.

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Bacteria produce surfactants with different surface activities and behaviours in air-water and oil-water mixtures. Surfactants

which reduce water surface tension the most are generally selected for biotechnological testing, but it is unclear how much structural and behavioural variation exists amongst these high-performing compounds. In order to investigate this question, a collection of 25 surfactant-expressing *Pseudomonas* spp. isolates producing a limited range of very low surface tensions (24 – 28 mN/m) in cell-free culture supernatants were examined. Analysis of the foam stability indices and oil-film displacement diameters (tested with mineral, vegetable, and used lubricating oil) suggest that there are significant differences in surfactant behaviour between the surfactant-expressing isolates (ANOVA, $P < 0.05$). This data was also used to construct a Hierarchical cluster analysis (HCA) dendrogram in which isolates were grouped according to similarities in surfactant behaviour (driven by inspection of the HCA scree plot and resulting in five additional non-expressing control isolates clustering together). Critically, this resulted in more groups (≥ 5 groups) than could be explained by statistically significant differences in mean surface tensions (≤ 2 groups as previously determined by ANOVA and Tukey Kramer HSD, *alpha* = 0.05). We interpret this to mean that this collection of *Pseudomonas* spp. isolates are expressing a number of structurally-different high-performing surfactants (i.e. different types) with varied air-water and oil-water behaviours. The HCA dendrogram produced using data from simple behavioural assays provides a useful tool to choose surfactants for future structural characterisation and testing for a range of potential biotechnology applications.

S17/P14

Association of antibiotic resistance profiles with plasmid architecture reveals potential novel drug targets.

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Excessive antibiotic use has led to an increase in antibiotic resistance amongst organisms found both environmentally and clinically. The genes encoding resistance are often plasmid-borne. Whilst the development of new antibiotics with traditional mechanisms has stalled, the transfer and replication elements of plasmids remain largely untargeted. The first step towards the use of these targets is to understand the architecture of the plasmids which encode resistance.

In this study, plasmids extracted from antibiotic-resistant *E. coli* isolated from a river receiving waste water effluent were characterised. Plasmid DNA was extracted and analysed using a combination of restriction enzyme mapping, multiplex PCR amplification of antibiotic resistance genes and identification of stability systems and incompatibility groups. Representative isolates were fully sequenced to determine the efficacy of these profiling techniques.

Through the use of these techniques, more than 200 plasmid isolates from resistant organisms were profiled revealing a number of groupings. The correlation of plasmid architecture and the associated resistance makers was explored. Additionally, we characterised the changes in environmental plasmid populations over a number of years to determine the persistence of different groupings. Future work will focus on the repurposing of drugs targeting replication and transfer elements as novel approaches to remove resistance-encoding plasmids from bacterial communities.

S17/P15

Molecular characterization and phenotyping diversity of *Cronobacter* spp. isolate from food sources.

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Cronobacter species are opportunistic food-borne pathogens that may cause life-threatening infections in neonates and infants. This study was conducted to investigate the phenotypic and genotypic diversity of *Cronobacter* spp. from food isolates. Fifty four strains have been used in this study. Most strains were motile except two *C. sakazakii* strains. *C. sakazakii* strains and 50% of *C. turicensis* strains were able to utilize sialic acid. Whilst most *C.sakazakii* and 70% of *C. dublinensis* strains were not able to utilize malonate . The five other Cronobacter species were able to utilize malonate. The majority of *C. sakazakii* strains had the most mucoid appearance indicating capsular material production. *C. sakazakii* strain (1888) showed dry colony appearance indicated no capsular material was produced. Moreover, considerable capsular material was produced by the six other *Cronobacter* species. All strains were able to form biofilms on plastic surfaces at 25°C and 37°C, although there was variation between strains with repeat the quantity. Strains were clustered into 43 sequence types and showed a high diversity according to multilocus sequence typing. The main serotype in *C. sakazakii* was CsakO:2 and serotype CmalO:1 was the main *C. malonaticus* serotype. There was a clear correlation between O-antigen serotyping and the values of capsule production. These results are an important contribution to an understanding of the diversity and characteristics of *Cronobacter* spp., which will help to identify contamination routes and the nature of persisting environmental strains and thus limit the risk for contaminations in food products, in particular, powdered infant formula.

S17/P17

pH profiles and survival of alkaliphilic floc forming anaerobic communities

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The aim of this investigation was to determine if floc formation is a survival strategy employed by alkaliphiles to attenuate the impact of hyperalkaline environments. This was determined by measuring the impact of environmental pHs >pH 11.0 on the

internal pH of flocs formed by an alkaliphilic bacterial community.

A stable, alkaliphilic floc based community has already been established at pH 11.0 from samples taken from a lime kiln waste site. Flocs from this community were incubated at pH 11.0, 11.5 and 12.0 for 1 hour. Internal pH profiles of these flocs were determined using a 10µm diameter micro pH probe attached to a micromanipulator. The profiles revealed the internal pH of the flocs to be lower than that found within the external environment, with minimum values of 10.41, 10.70 and 11.62, respectively. Subcultures of these flocs were grown at pH 11.0, 11.5, 12.0 and 13.0 over 3 weeks with microbial growth measured via ATP detection. The results demonstrated that these flocs were able to grow at pH 11.0 and 11.5 and could survive at pH 12.0 but not pH 13.0.

Alkaliphilic flocs are able to maintain an internal pH that is approximately 0.5 pH unit lower than the pH of the external environment. This allows the associated microbial community to survive in environments with a pH up to pH 12.0. This observation provides some explanation for the presence of active alkaliphilic communities at pH values considered to be thermodynamically unfavourable for microbial life.

S17/P18

Assimilation of rhizosphere colonization by *Bacillus amyloliquefaciens* FZB42 and *Bacillus subtilis* BBG131 as influenced by different factors

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Bacillus amyloliquefaciens FZB42 is a wild-type strain, produces three families of lipopeptides (fengycin, surfactin and bacillomycin) and shows a high potential as biopesticides for the control of plant diseases whereas, *Bacillus subtilis* BBG131 is a genetically engineered microorganism which overproduces surfactin. Many of the two species are belonged to PGPR.

At this study different sugars and organic acids which are naturally found in the rhizosphere as a major composition of root exudates, were used as soles substrates for the bacterial growth. The concentrated root exudates were also used for the same purpose. The lipopeptides production as influenced by different carbon sources was quantified.

The bacterial growth kinetic was different and it was depending on the substrates. Glucose was the best carbon sources for the two strains, whether for the growth or lipopeptides production. no lipopeptides production and low growth of the two strains was observed in the presence of oxalic acid as a sole carbon source. Fructose and xylose were showed a high different on surfactin production. Plant growth of tomato expressing as dray weight of plant and length of stems, was more with *B. amyloliquefaciens* FZB42 than *B.subtilis* BBG131.

As a conclusion, the different behavior of the ability of rhizosphere colonization may be due to the distinct behavior of the two strains to consume the root exudates or the differences carbon sources in the root exudates which is found in the rhizosphere and thus it is reflex to the ability to colonize the rhizosphere.

S17/P20

Characterization and Classification of Bacteriocins produced bySelected Lactic Acid Bacteria Isolated from Fermented African Alcoholic Beverage (Pito)

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Characterization and Classification of Bacteriocins produced bySelected Lactic Acid Bacteria

Characterization and classification of bacteriocins produced by selected lactic acid bacteria (LAB) isolated from fermented African alcoholic beverage (Pito) was carried out. TheLactobacillus acidophilus PIT 17 and Leuconostoc mesenteroides PIT 11 were isolated using the pour plate technique and were propagated in De Man rogosa Sharpe (MRS) broth separately for bacteriocins production. Differences in total activity, specific activity, protein contents and bacteriocin activity during the bacteriocin purification processes were observed. After the purification stages of bacteriocins produced, Lactobacillus acidophilus PIT 17 had higher bacteriocin recovery (19.15%) while Leuconostoc mesenteroides PIT 11 (18.0%) bacteriocin recovery. The bacteriocins produced by the bacteriocin producing LAB inhibitedPseudomonas aeruginosa, and Staphylococcus aureus. Bacteriocin produced byL. acidophilus PIT 17 and L. mesenteroides PIT 11 exhibited bacteriocin activity of 8400±0.00 and 8000±0.00 respectively. The bacteriocin produced by L. acidophilus PIT 17and L. mesenteroides PIT 11 were stable, slightly stable and unstable at various exposed,storage teperatures and at different pH levels. The bacteriocins produced by L. acidophilusPIT 17 and L. mesenteroides PIT 11 were identified as acidophilin belonging to class III bacteriocin and mesentericin belonging to class II bacteriocin respectively. Generally,bacteriocins enhances biopreservation of food products when used as biopreservative.

S17/P21

In vitro antimicrobial Inhibitory effects of Eugenol on the growth of medically important pathogenic organism

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Although pharmaceutical companies and researchers have developed a number of new antimicrobial agents during the last

decades, resistance to these agents have been commonly reported. Therefore, the prevention of infection using natural based approaches is desirable and the potential effect of natural options for long-term prevention of infections should be explored. Hence, in this study we examined the in vitro antimicrobial activities of Eugenol, the main chemical component of clove oil against medically important pathogens. To assess the antimicrobial properties of Eugenol, nine different ATCC strains of bacteria which represent gram positive and gram negative medically important pathogenic are used through this study. The antimicrobial activity of the Eugenol was determined using the agar-well diffusion method. The in vitro antimicrobial activity was performed using the agar-well diffusion method of the Eugenol and resulted in a range of different ranges of growth inhibition pattern against tested microorganisms. Eugenol showed an inhibitory zone diameter of 2–23 mm against all the pathogenic bacteria. The less activity against the tested microorganisms is found especially against *Proteus mirabilis* and *Acinetobacter baumannii*, whose zones of inhibition ranged from 2 mm to 12 mm. The largest diameter (23 mm) was observed with on *Escherichia coli* and *Klebsiella pneumoniae* and those strain were considered to be susceptible to eugenol. The oil being active against the isolates is an indication that it can be a source of very potent antibiotic substances that can be used against multidrug resistant microorganisms

S17/P22

Paper title: isolation and characterization of novel alkaliphilic bacteria from soil contaminated with lime kiln wastes.

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Abstract
Lime kilns have operated at various scales in the UK since the 16th century, leaving a legacy of alkaline contaminated soils. One characteristic of these sites is that cellulosic materials in the soil may undergo anaerobic, alkaline hydrolysis to generate a range of small organic molecules (cellulose degradation products or CDP) that are amenable to microbial degradation. Batch fed microcosms inoculated with soil from a lime kiln waste site and fed on CDP were established at pH 10.0 and used as a source of novel alkaliphiles. These isolates were grown anaerobically on solid media using CDP and the calcium salt of iso-saccharinic acid as sole carbon sources. Individual isolates were identified via 16S rRNA gene sequencing and subjected to pH profiling and heavy metal resistance assessment using the Bioscreen automated growth system.

As would be expected bacterial community analysis indicated a significant drop in diversity between the initial soil and the stabilised microcosm (>12 months operation), despite the fact that Firmicutes and Proteobacteria were the dominant phyla in both cases. A wide range of isolates were established from this microcosm, these were primarily Gram positive, facultative anaerobes with an upper pH limit of pH 10.0. A smaller number of isolates were able to grow up to a pH of pH 11.0. Many isolates generated extracellular polysaccharides and demonstrated a range of tolerances to heavy metals such as Ni, Cu, Cd, Pd and Co. Isolates chosen for further investigation are from the following genera: *Macellibacteroides*, *Shewanella*, *Dietzia* and *Tessaracoccus.*

S17/P23

Microbial Community Structure of a Low Sulfate Oil Production Facility and The Effects of Substrate availability and Environmental Factors on Methanogenesis and Corrosion rates

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Analysis of microbial community structure of a low sulfate oil producing facility in Nigeria using 16S rRNA gene sequencing technique revealed dominance of oil degrading and nitrate reducing bacteria and methanogenic archaea in produced waters and oil samples (Betaproteobacteria 33-42%, Marinobacter 37%, Azovibrio 21%, Thauera 10-28%, and Methanolobus 22%). On the contrary, the associated oil pipeline samples revealed massive dominance of potentially corrosive Methanolobus (60%) and Methanobacterium (25-27%). Further experimentation shows that the methanogens implicated in oil pipelines are moderate halophytes and utilizes H2/CO2 and methanol as substrates. The methanogens also produced methane over a wide pH range and at moderate temperatures and methane production correlated strongly with general corrosion rates under different conditions of substrate availability and environmental factors tested. More emphasis should be on methanogenic archaea as opposed to sulfate reducing bacteria (SRBs) during mitigation plans for microbially induced corrosion (MIC) in a low sulfate oil producing facility.

Keywords: Low sulfate oil facility, Oil degrading bacteria, Nitrate reducing bacteria, Methanogens, MIC

S17/P24

Quorum sensing in industrial fermentation: Characterizing solvent producing *C. autoethanogenum*

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Clostridium autoethanogenum is a Gram-positive, motile and anaerobic bacterium. The rod-shaped organism was first discovered and isolated from rabbit faeces and is capable of autotrophic growth by fixing carbon in the form CO and CO2. Acetogenic organisms such as *C. autoethanogenum* share the Wood-Ljungdahl pathway, which is integral to carbon fixation, and producing a variety of fuel-viable solvents.

Quorum sensing (QS) is cell to cell communication is responsible for concerted, population-wide changes in gene expression and

behaviour in response to cell population density. QS has not fully been understood in *Clostridium autoethanogenum*, and progress has been made to delete components of several putative agr signalling systems, resulting in a set of knock out mutants. It is hypothesised that quorum sensing might be responsible for sporulation initiation, as shown for other clostridial species. It may also play a role in regulating fermentation metabolism as shown for the related but non-acetogenic *Clostridium acetobuytlicum.* By using the in-frame gene deletion methods, three separate agrD mutants have been created; agrD1, agrD2, agrD1D2. These agrD permutations will allow in-depth study of this system, and experiments alluding to phenotype changes will be done to note the differences between mutants and wild type variants.

S17/P25

Deterministic assembly processes governing bacterial community structure in the Fynbos, South Africa

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The Mediterranean Fynbos vegetation of South Africa is well known for its high levels of diversity, endemism, and the different existence of distinct plant communities on different soil types. Studies have documented the broad taxonomic classification and diversity patterns of soil microbial diversity, but none looked at the community assembly processes. We hypothesised that bacterial phylogenetic community structure in the Fynbos will be highly governed by deterministic processes. We sampled soils in four Fynbos vegetation types and examined bacterial communities using Illumina HiSeq platform with the 16S rRNA gene marker. The overall phylogenetic signal indicates distantly related OTUs tend to co-occur and only a small proportion of closely related OTUs were ecologically coherent. However, UniFrac analysis showed that the community clustered strongly by vegetation types, suggesting a history of evolutionary specialisation into certain vegetation types. The standardised beta mean nearest taxon distance (ses. MNTD) index, showed no association with vegetation type. Both NTI (nearest taxon index) and ses.MNTD significantly deviated from the null models, indicating that deterministic processes were important in the assembly of bacterial communities. Furthermore, ses.MNTD was significantly higher than null expectations, indicating that over-dispersion in phylogenetic beta diversity is explicable by the differences in environmental conditions across the sites. Overall, this study sheds light on the relative roles of both deterministic and neutral processes in governing bacterial communities in the Fynbos. It seems in part that deterministic processes play a major role in assembling bacterial community, and neutral processes playing significantly minor role.

S17/P27

Assimilation of rhizosphere colonization by *Bacillus amyloliquefaciens* FZB42 and *Bacillus subtilis* BBG131 influenced by different factors

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Bacillus amyloliquefaciens FZB42 is a wild-type strain, produces three families of lipopeptides (fengycin, surfactin and bacillomycin) and shows a high potential as biopesticides for the control of plant diseases whereas, Bacillus subtilis BBG131 is a genetically engineered microorganism which overproduces surfactin. Many of the two species are belonged to PGPR.

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The bacterial growth kinetic was different and it was depending on the substrates. Glucose was the best carbon sources for the two strains, whether for the growth or lipopeptides production. no lipopeptides production and low growth of the two strains was observed in the presence of oxalic acid as a sole carbon source. Fructose and xylose were showed a high different on surfactin production. Plant growth of tomato expressing as dray weight of plant and length of stems, was more with *B. amyloliquefaciens* FZB42 than *B.subtilis* BBG131.

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S17/P30

Characterization of antibiotic resistant bacteria and detection of multi-drug resistance plasmids in wastewater treatment plant effluent in Ireland

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Wastewater treatment plants (WWTPs) have been recognized as reservoirs of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). WWTP effluent was characterized in Ireland with respect to ARB and the presence of plasmids mediated ARGs (R-plasmids). Samples from two WWTPs were

collected in March and October 2015 and screened for the prevalence of amoxicillin, ciprofloxacin and tetracycline resistance in total faecal coliforms and enterococci. The minimal inhibitory concentrations of these bacteria were determined for nine antibiotics from seven different classes. R-plasmids were isolated both from the cultured bacteria and using an exogenous method to isolate plasmids directly from WWTP effluent samples. Among total faecal coliforms, the most prevalent resistance phenotype in WWTP A and B, respectively, was observed for amoxicillin (27.4%, 41.6%), followed by ciprofloxacin (12.5%, 8.9%) and tetracycline (4.8%, 3.2%). Amoxicillin resistant enterococci were not identified in any samples. The prevalence of tetracycline resistance in enterococci were 6.8% and 7.1%, and ciprofloxacin resistance 4.3%, 3.4%, in each sample respectively. More than 30% of all selected isolates were multidrug resistant. Twenty five R-plasmids were identified from these cultured bacteria and ten directly from the samples. R-plasmids will be analysed further to understand the mechanisms of the ARGs present on the plasmids and the similarities between the plasmids. The presence of ARB and R-plasmids in WWTP effluent demonstrated that WWTP contribute to the dissemination of ARB and ARGs in the environment, which could impact human health.

S17/P31

Role of the pentose phosphate pathway in free-living and symbiosis in Sinorhizobium meliloti

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Sinorhizobium meliloti is a Gram-negative α-proteobacteria which is capable of entering into a symbiotic relationship with *Medicago sativa*, and has the ability to catabolise a wide variety of carbon substrates. While characterizing galactose catabolism in *S. meliloti*, it was found that mutants unable to utilize galactose as a sole carbon source acidified the medium and overproduced succinoglycan (EPS-I). The overproduction of EPS-I was due to an acidification of the medium, and subsequent work showed that EPS-I allowed the bacteria to survive in a low pH medium.

To determine what contributed to medium acidification, a mutant unable to catabolise galactose was mutagenized with Tn*5* and screened for mutants unable to acidify the medium. A mutant unable to acidify the medium was isolated and found to carry a transposon in the gene *tkt2*, a putative transketolase. A characterization of the *tkt2* mutation showed that it had a decreased production of EPS-I, was sensitive to low pH, and had an aromatic amino acid auxotrophy, suggesting that this transketolase was a part of the pentose phosphate pathway. Since the pentose phosphate pathway is poorly characterized in *S. meliloti*, we constructed a transaldolase mutation (*tal*). With strains that have mutations in genes that encode the two key enzymes of the pentose phosphate we are characterizing the role the pentose phosphate plays in affecting the overall free-living and symbiotic states of *S. meliloti.*

S17/P32

Identification of Microorganisms at Species Level Using Rapid Evaporative Ionisation Mass Spectrometry (REIMS)

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The introduction of mass spectrometry to clinical microbiology laboratories has revolutionised their work flows, and substantially reduced diagnosis times. However, currently used matrix-assisted laser desorption ionisation time of flight (MALDI-ToF) platforms require the addition of a matrix to aid ionisation, and relies upon the isolation of pure microbial cultures before analysis. Further, MALDI-ToF platforms are only semi-automated, and are limited in their ability to differentiate certain bacterial species, such as *Salmonella sp.*, and in sub-species analysis. Rapid evaporative ionisation mass spectrometry (REIMS) has previously been shown to allow the differentiation of bacterial and fungal species, based upon their lipidomic profiles, directly from pure microbial colonies. This initial work utilised monopolar and bipolar forceps to analyse microbial colonies grown on agar plates. We are currently optimising the REIMS platform to provide an automated analysis platform aided by a robotic system, and exploring the effectiveness of running solvents to improve analyses. Work is currently underway to construct a REIMS mass spectra library of approximately 50,000 isolates from 4,000 species; which will also serve as a substantial microbial lipidomic and metabolic database. This mass spectra library will be utilised in the identification of microbial isolates without the preparative steps required for MALDI-ToF. Furthermore, this mass spectra library will allow the effectiveness of the REIMS platform to assign taxonomic classifications to mixed microbial communities to be determined. This presentation will give a background to the REIMS platform and its potential application to clinical microbiology laboratories and beyond.

S17/P33

Development of a Class-Research Project: Beyond One Semester

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The University of Puerto Rico at Humacao Campus started a collaborative project, as part of the Small World Initiative (SWI) with two main objectives: to establish a community of undergraduates researchers in the classroom and to screen the soil tropical microbiota that are able to produce compounds that control the growth of other microorganisms. To attract talented students that are not engage in a formal research project we

implemented the SWI curricula in the Cell and Molecular Laboratory (CML), the Biotechnology (B) course and independent study group (ISG). An active learning communities, that goes beyond the semester was established. The CML community isolated a total of 9 antimicrobial producers bacteria during the course of the fall semester and now these bacteria are being further study by the B and ISG groups. Students isolated their bacterial from different type of tropical soils and they were able to design their own experiments, consequently, each group in the classroom was working, not only in different isolates but also using different techniques and bioassays. A rubric was prepared by participating faculty and offered as a pre- and post-test that measures computational biology skills with emphasis in several areas: graphical analysis, growing bacteria, identifying antibiotic production, and applying microbiology data to a practical problem. Results showed a gain (a Hake's Gain rise up to 57%) in five from the nine tested areas. An active learning environment has being fostered throughout these courses and also through other activities.

S17/P34

Isolation of non-solvent producing degenerates of Clostridium beijerinckii NCIMB 8052 and the mechanisms for the occurrence of this phenomenon

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Concerns about global warming have led to a renewed shift in focus towards the production of biofuels as an alternative to fossil fuels. Solventogenic Clostridia offer an attractive alternative to produce biofuels, specifically biobutanol, which can be substituted for traditional gasoline in vehicles. An intrinsic problem with solventogenic Clostridia, however, is their spontaneous degeneration which typically occurs when the bacteria are repeatedly sub-cultured in batch culture or grown in continuous culture. The phenomenon of degeneration in solventogenic species is characterised by the inability to produce solvents and form spores. Degenerates also display different colony morphologies to their wild type counter parts. At present, there is no definitive or universal cause for degeneration. However, several mechanism including acidification of the media and defects in quorum sensing have all been suggested. To gain a further understanding of the degeneration process, Clostridium beijerinckii NCIMB 8052 was repeatedly sub-cultured in liquid media and subsequently grown on solid agar media in an attempt to isolate potential degenerates. Once suspected degenerates were identified, based on changes in colony morphology, their solvent producing capability and cell physiology were determined. For confirmed degenerates showing reduced or abolished solvent production and sporulation, whole genome sequencing was carried out to allude to any genomic changes in comparison to the parental strain.

S17/P35

Identifying infection reservoirs of digital dermatitis in dairy cattle

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Bovine digital dermatitis (DD) is an infectious ulcerative disease affecting the skin on the hind feet of dairy cattle. The disease is endemic in the UK and found worldwide. DD is a painful disease, often causing lameness, and is of economic importance to the dairy industry. The causative agents are considered to be spirochaetes belonging to the genus *Treponema*, however, little is known about the transmission or infection reservoirs of these DD associated treponemes. The aim of the study was to better understand the relationship between the dairy cow, the farm environment and DD treponemes by conducting DD treponeme survival studies in bovine faeces. Three phylogroups of DD treponemes were inoculated into faecal microcosms and incubated at 12oC for 7 days. On each day of the 7 days, a small sample of the faecal microcosm was inoculated into selective medium for secondary culturing. Growth and motility of the three phylogroups of DD treponemes was monitored by phase contrast microscopy. All three phylogroups of DD treponemes appeared to neither grow, nor decrease in prevalence throughout the faecal incubation period. Faeces did not appear to inhibit nor aid the subsequent growth of two of the DD treponeme phylogroups in the secondary cultures; however, faeces did appear to inhibit the subsequent growth of one of the phylogroups in secondary culture. Knowledge of DD treponeme survival in faeces will provide a better understanding of the role of faeces as a potential infection reservoir and will impact upon farm biosecurity.

S17/P36

The effect of coinoculaton with *Bradyrhizobium japonicum* USDA 110 and *Pseudomonas putida* NUU8 on nitrogen and phosphorus availability to soybean growth

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We studied the effect of *Bradyrhizobium japonicum* USDA 110 alone and in combination with *Pseudomonas putida* NUU8 on nutrient contents of nitrogen and phosphorous uptake efficiency of soybean (*Glycine max* L.) plants grown under hydroponic conditions in a greenhouse. Plants were grown for 45 days in greenhouse with an average temperature of 23/20oC (day/night), and relative humidity of 48/83% v/v (day/night). At harvest, soybean shoots, roots and nodules were separated. Shoots, roots and nodules were oven-dried to a constant weight at 75oC for 48

hours. The results showed that when soybean was inoculated with *B. japonicum* USDA 110 strain alone up to 25% of the amount of root N content, the amount of P in the root and shoot the amount of N and P content increased to 20% compared to control. When the symbiont *B. japonicum* USDA 110 was co-inoculated with *P. putida* NUU8, the nitrogen and phosphorus contents of soybean were as significantly increased compared to uninoculated. The increase in nodule P and N contents was significant for soybean grown in Low Nitrogen and Low phosphorus solutions and co-inoculated with *B. japonicum* USDA 110 and *P. putida* NUU8 compared that plants inoculated with *B. japonicum* USDA 110 alone. According to the analysis of the results obtained, giving a soybean processing plant *B. japonicum* USDA110 food nutrition has improved compared to the control elements of the shadows. Especially soybean plants co-inoculated with *B. japonicum* USDA 110 and *P. putida* NUU8 strains N, P nutrients were found to be relatively high.

S17/P37

Enhancement of nodulation, plant growth and nutrient contents of soybean by co-inoculation with rhizobacteria

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We studied the effect of *Bradyrhizobium japonicum* USDA 110 alone and in combination with Pseudomonas putida NUU8 on nodulation, plant growth, and nutrient contents of soybean (*Glycine max* L.) in pot experiments under greenhouse conditions. Plants were grown in loamy sandy soil taken from ZALF experimental field station, Müncheberg, Germany. Plant growth parameters such as root and shoot length, dry biomass, nodulation, and physiological properties (N, P, K, Na, Mg, and Ca) were measured. The results showed that a combinated treatment of *B. japonicum* and *P. putida* significantly increased the nodule number per plant, the length of roots and shoots, and root and shoot dry weights under greenhouse conditions. The significant increases in nodule number and in root and shoot dry weights on co-inoculation of *P. putida* strain NUU8 with *B. japonicum* strain USDA 110 were due to direct and indirect enhancement of plant growth by a variety of mechanisms such as fixation of atmospheric N, solubilization of minerals such as P, synthesis of phytohormones (direct mechanism) and the ability to induce systemic resistance of plant growth–promoting rhizobacteria (indirect mechanism).The maximum significant increase in nutrient contents were recorded after co-inoculation of *B. japonicum* USDA 110 and *P. putida* NUU8 compared to uninoculated control. Hence, co-inoculation with *Bradyrhizobium japonicum* and *Pseudomonas putida* may be effective as indigenous plant growth promoting rhizobacteria for soybean production systems.

S17/P38

EFFECT OF ENCAPSULATION ON THE VIABILITY OF PROBIOTICS IN YOGHURT

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It is well entrenched that probiotic bacteria exert myriad of beneficial effects on human health, including antibiotic therapy, improved symptoms of lactose intolerance, resistance against cancer, reduced incidence of diarrhea in humans and production of antimicrobial substances and reducing cholesterol level. The intension of this study was to evaluate the stability of Probiotic in the yoghurt with and without encapsulation. Probiotic yoghurt was compared with control yoghurt in terms of chemical, physical, microbial and sensory properties over a period of 15 days of storage. Yoghurt was prepared with free lactic acid bacteria and with encapsulated bacteria and was stored at 4 0C. Yoghurt was subjected to physiochemical and microbial analysis. The addition of the probiotic bacteria in the yoghurt samples either in encapsulated or without encapsulation significantly affected the results for pH, lactose, acidity, viscosity and syneresis. However, the addition of the strains enhancing probotic production either free or encapsulated form did not bring any discrete difference in color, flavor and taste over the 15 days of storage period.

S17/P40

Fengycin regulation: between the promoter expression and gene transcription responding to different culture conditions.

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Non-ribosomally synthesized lipopeptides are one of the main groups that are produced by *Bacillus* spp. Fengycin is one of these lipopeptide families known to have a strong antifungal activity. GFP protein was inserted under the control of the fengycin promoter in the overproducer strain called BBG21 (spontaneous mutant strain from *Bacillus subtilis* ATCC 21332) generated the strain BBG205. In this study we used this marker to study the effect of the different medium compositions on the fengycin promoter expression in order to understand the regulation interact between the nutrients metabolism and the fengycin synthesis, as well as the reflex of the promoter

expression on the fengycin gene translation. The results showed that using different nitrogen and carbon sources influence strongly on the fengycin promoter expression. Two types of conditions were observed: promoter activator condition and production activator condition. The urea and starch were the best nitrogen and carbon source respectively, as promoter activator condition, while the mixed of urea and ammonium carbonate as nitrogen source and mannitol as carbon source gave the highest production levels. Using the mannitol or the urea as promoter activators make that possible to activate the promoter by definite substrate conditions then feeding the culture by another preferable cell growth source (sucrose or glucose with our strain) to obtain high production amount of fengycin. This strategy enabled us to increase the fengycin production more than 2-fold with many sources to achieve until 768 mg/L as the highest production had been reported from this strain.

S17/P41

The LacI Family Transcription Regulator, RbsR, Co-regulates the Production of Gas Vesicles and Secondary Metabolites in *Serratia*

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Serratia sp. ATCC39006 (*S*39006) is a Gram-negative, motile, rod-shaped bacterium. It is the only enterobacterium known to make gas vesicles (GV) naturally. GVs are proteinaceous, intracellular organelles used by bacteria to enable upward flotation in aquatic environments. The production and regulation of GV assembly is a complex process, which is likely part of a wider regulatory network affecting various phenotypes, including secondary metabolite biosynthesis. This study aims to identify new genes involved in the co-regulation of GV production and biosynthesis of secondary metabolites, such as carbapenem and prodigiosin antibiotics. To identify new regulator genes, a comprehensive mutant library containing 58,739 insertion mutants was first generated by random transposon mutagenesis. In addition to insertions within the known GV production gene cluster, 9 other genes were identified as involved in GV regulation. One of these GV-defective mutants had a transposon inserted in the LacI family transcription regulator gene (*rbsR*) of the ribose operon. Transmission electron microscopy images confirmed that the *rbsR* mutant did not produce GVs. In addition, this mutant was defective for production of two structurally-unrelated antibiotics; a carbapenem and prodigiosin, but it exhibited increased swimming and swarming motility. Therefore, RbsR is not only important for the regulation of GV formation, but it also has complex pleiotropic physiological impacts on the production of two bioactive secondary metabolites.

S17/P42

Evolution of Pseudomonas fluorescens motility.

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Pseduomonas fluorescens strain SBW25 is known to express a number of different motile phenotypes. Little is however known of the mechanisms and selective forces behind these adaptations. One potential selective force could be resource availability. For example, it has been recently shown that active nitrogen regulation can aid in the evolution of motility with non-motile mutants of *P. fluorescens*. I progress on this research by using experimental evolution to study how nutrient environment affects the evolution of motility and diversification of different motile phenotypes. I found that P. fluorescens rapidly diversifies into different motile phenotypes depending on the resource environment and selection for motility (positive vs. negative selection); Spidery, Smooth and a novel 'sun-like' phenotypes. The spidery phenotype evolved only in M9 media, which suggests increased flagellar number selected by nutrient starvation (hypermotility). The smooth phenotypes evolved in the abundance of nutrients (LB media) and generally under a positive selection for motility. This form of motility could be due to biosurfactant expression, namely viscosin, which aids flagellar based motility across a surface. The 'sun-like' expression occurred only under negative selection for motility in LB media. Interestingly, non-motile *fleQ* knock-out SBW25 mutants did not diversify into distinct phenotype, even though their motility increased during the selection experiment. This suggests that *fleQ* may play a role in the expression of the diverse SBW25 phenotypes. Future research will try to identify the underlying mechanisms under these adaptations.

S17/P43

Genome mining and phenotype microarray analysis of lead (Pb)-dependant antibiotic producing Streptomyces spp.

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There are more than 22,000 known microbial secondary metabolites, 70% of which are produced by actinomycetes and half of these are produced by streptomycetes bacteria (Subramani & Aalbersberg, 2012). Therefore, streptomycetes are widely recognised as pharmaceutically important microorganisms. However, much of the biosynthetic potential of these microorganisms is not observed under standard laboratory conditions.

Bioinformatics analysing of the complete genome sequences of the some model Streptomyces strains have revealed the presence of over 25 biosynthetic gene clusters some of them without a known metabolic product that might be involved in the biosynthesis of new secondary metabolites belonging to different structural classes (Olano et al., 2014).

Here we have sequenced a terrestrial Streptomyces isolate which produced un known bioactive metabolites under lead metal induction using Ion Torrent (PGM) next generation sequencing technology for genome mining as a new strategy for natural products discovery and typing novel bacterial isolates. Moreover, genomic features were combined and correlated with phenotype microarray assay using biology plates under different growth conditions.

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S17/P44

The role of fibrobacters in the landfill cellulolytic microbial community

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Despite the abundance of cellulose in landfill sites, there is limited understanding of the taxonomy and function of the cellulolytic microbial community in this environment. Here, we compared the biofilms of poorly and heavily degraded colonised cotton 'baits' incubated in landfill leachate using 16S rRNA gene PCR amplicon sequencing, shotgun metagenome sequencing and Scanning Electron Microscopy. A total of 22 phyla were detected by 16S rRNA gene amplicon sequencing of poorly degraded cotton, including *Bacteroidetes* (72.7%), *Proteobacteria* (18.7%) and *Actinobacteria* (3.3%). In contrast, 24 phyla were detected on heavily degraded cotton, with *Firmicutes* (34.7%), *Bacteroidetes* (20.5%), *Spirochaetes* (14.8%) and *Fibrobacteres* (14.2%) predominating, implicating these taxa as key members of the cellulolytic community in landfill sites. Additionally, anaerobic isolation and cultivation of bacteria present on heavily degraded cotton yielded 55 isolates that were either *Clostridium* spp. or contained a mixture of clostridia and fibrobacters. Metagenome sequencing of the heavily degraded cotton biofilm identified 18 phyla, including members of the *Firmicutes* (47.2%), *Bacteroidetes* (22.8%) and *Spirochaetes* (6.1%), whilst also potentially implicating Proteobacteria (6.4%) and *Fibrobacteres* (0.8%) as having a role in cellulose degradation in landfill sites. Recruitment plot analysis of the heavily degraded colonised cotton metagenome also implicated members of the *Fibrobacteres* in cellulose hydrolysis, with the genome of *F. succinogenes* subsp. *succinogenes* S85 having the most protein matches with the metagenome. These data provide important insights into the ecology of cellulose hydrolysis in anaerobic environments, with implications for our understanding of global carbon cycling and waste management.

S17/P46

In vitro testing of Kunzea, thyme and tea tree oils to the mastitis causing pathogens Staphylococcus aureus, Streptococcus uberis and Escherichia coli

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Mastitis costs the UK agricultural industry approximately £200 million pa, though lost yield, culling and veterinary treatments. Concerns about downstream effects such as antibiotic resistance have increased interest in alternative treatments and a number of commercially available products containing essential oils can be used by organic farmers or those wishing to reduce antibiotic costs. Thyme oil and tea tree oil have been shown to have antibiotic action on a range of pathogens, while kunzea oil is less well known. These were tested *in vitro* on *Staphylococcus aureus*, *Streptococcus uberis* and *Escherichia coli*, all indicating antibiotic action. Kunzea and thyme were most effective against *S. aureus* and *S. uberis* with thyme oil most effective overall. Further research will include testing these in combinations and identifying Minimum Inhibitory Concentrations, with a view to identifying potential for replacing or supplementing conventional antibiotic treatments.

S17/P48

Purification and Characterization of Alkaline lipase From *Brevundimonas diminuta* Isolated from Cocoa Processing Plant Effluent

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Implications Effluent from cocoa-processing plants constitutes one of the sources of oily waste in the environment. This oily effluent being discharged serves as source of lipase producing microorganisms. Hence, this study screened lipase-producing bacterium from the effluent as well as characterized the partially purified lipase produced by the isolated bacterium for lipase production.

Methods The bacterium with the highest lipase activity was selected and identified using 16S rRNA gene sequencing technique. Lipase activity was assayed by spectrophotometric method. The enzyme was partially purified by ion-exchange chromatography using CM-Sephadex C-50 and was characterized for activity based on temperature, pH, metal ion and organic solvents. The kinetic parameters of the purified enzyme were also studied.

Results The bacterium with the highest lipase activity was confirmed to be *Brevundimonas diminuta*. The organism utilized the effluent (with 4.78 ± 0.16 U/ml) as carbon source. The specific activity of the partially purified lipase was 260.3 Units/mg protein. The lipase had a molecular weight of 60 kDa. The enzyme was found to be alkaline tolerant with optimum activity at pH 8.0.The enzyme activity was found to be stable with maximal activity at 45oC. The enzyme retained about 70 % of its activity at 45°C after 1h. Lead and Barium enhanced the lipolytic activity of the enzyme. However, mercury significantly inhibited the enzyme activity. The Michaelis constant was found to be low which indicates strong affinity of the enzyme for its substrate p-Nitrophenolpalmitate.

Conclusion *Brevundimonas diminuta* produced an alkaline lipase which could be suitable for industrial and biotechnological applications.

S17/P49

Activities of amylase, proteinase, and lipase enzymes from *Lactococcus chungangensis*

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Amylase, proteinase, and lipase are enzymes that are found in milk and play an important role in degrading milk into monomeric molecule such as oligosaccharides, amino acids, and fatty acids, which are the main molecules responsible for the flavors in cheese. In the current study, the amylase, proteinase, and lipase activities of *Lactococcus chungangensis* CAU 28T, a bacterial strain of non-dairy origin, were determined in bacterial culture, cream cheese, and yogurt and were compared with those of the reference strain, *L. lactis* subsp. *lactis* KCTC 3769^T that is commonly used in the dairy industry. *L. chungangensis* CAU 28^T and *L. lactis* subsp. *lactis* KCTC 3769^T were both found to have amylase, proteinase, and lipase activities in broth culture, cream cheese, and yogurt. Notably, the proteinase and lipase activities of *L. chungangensis* CAU 28^T were higher than those of *L. lactis* subsp. *lactis* KCTC 3769^T, with proteinase activity of 10.50 unit/ml per gram in tryptic soy broth and 8.64 unit/ml per gram in cream cheese, and lipase activity of 100 unit/ml per gram of tryptic soy broth and 100 unit/ml per gram of cream cheese. In contrast, the amylase activity was low, with 5.28 unit/ml per gram in tryptic soy broth, 8.86 unit/ml per gram in cream cheese. These enzyme activities in *L. chungangensis* CAU 28^T suggest that this strain has potential to be used for manufacturing dairy fermented products, even though the strain is of non-dairy origin.

S17/P50

Human Scalp Microbiome Dynamics in Exacerbations of Dandruff

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Dandruff is a common problem globally with a prevalence of up to 50% in both genders. However, although there are known bacterial and fungal associations with dandruff, an understanding of the etiology of dandruff is incomplete. To investigate the bacterial and fungal communities associated with dandruff, eighteen samples were collected from the scalps of healthy controls and those with moderate and severe dandruff. The variable regions V1-V3 of the 16S rRNA gene (bacteria) and the D1/D2 region of the 26S rRNA gene (fungi) were amplified using universal primers. A total of 155,378 16S rRNA and 26S rRNA gene sequences were obtained using GS-FLX pyrosequencing. Firmicutes were the most dominant species in scalps with dandruff, increasing from 5.2% in cases defined as normal to 91.0% in those with severe dandruff, whereas Proteobacteria decreased from 60.1% in normal scalp samples to 6.2% in dandruff samples. Fungi of the phylum Basidiomycota were associated with the normal scalp, decreasing from 66.6% to 24.7% in cases of severe dandruff. In the phylum Firmicutes, *Staphylococcus* spp. were dominant in the dandruff scalp. In contrast, *Malassezia* spp. in the phylum Basidiomycota were the most abundant fungus in the healthy scalp. This switch from *Malassezia* to *Staphylococcus* provides new comparative information on the microbiomes of the normal scalp and those with exacerbated dandruff. Consequently, these results can be expected to lead to improved diagnosis and treatment.

S17/P51

Bioremediation of benzene, toluene and xylene using immobilized fungi isolated from oil-contaminated areas

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Benzene, toluene, and mixture of xylenes (BTX) represent an important class of environmental contaminants. Bioremediation is an economical, efficient, and environmentally friendly approach used to promote their removal and treatment of contaminated areas. The present work aimed to select marine fungal strains, isolated from oil-contaminated areas in Santos (Brazil), and able to biodegrade these compounds.

Several fungal strains were isolated using solid media, followed by biodegradation experiments (using free and immobilized cells in alginate), which were carried out in liquid media supplemented with BTX for 30 days, at 30 °C and 150 rpm. Growth profiles and BTX biodegradation were determined using dry weight and chromatography analysis, respectively.

As a general trend observed in all tested strains, immobilized cells displayed increased BTX degradation rates when compared with free cells. The best results were obtained with immobilized cells of two fungal strains (CLP-01 and CLP-05), which showed the highest values of biomass and benzene (75%), toluene (82%) and xylene (90%) degradation. These strains will be further studied, as they show significant potential for application in bioremediation of BTX, and treatment of contaminated areas.

S17/P52

The treatment of bacterial disease of plants by bacteriophage-coated nanoparticles

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Bacterial phytopathogens are a recurring issue for agricultural plants. Traditional control measures include copper treatment and use of pesticides and antibiotics. The use of bacteriophages that selectively kill the causative pathogen as an alternative treatment has been delayed by technical difficulties associated with phage stability and deployment methods. In this project, we will develop phage-coated nanoparticles for the control of tomato plant soft rot, caused by *Pectobacterium carotovorum*, as a model system. Scottish crops were used as a source to isolate new bacteriophages, followed by triple isolation of single plaques formed after infection. Using the enrichment technique, that uses bacteria to isolate the virus from a soil sample, 13 potentially different bacteriophages were recovered and their characterisation by genome sequencing, is underway. So far all of the phages characterised resemble T7. In addition we also characterised the phages morphology by transmission electron microscopy; consistent with the genomic characterisation, electron microscopy revealed that the phages displayed a morphology characteristic of the *Podoviridae*. Subsequently, we will covalently bind a collection of phages to the surface of nanoparticles synthesised from a wide variety of materials and the capacity of these phage coated nanoparticles to control bacterial plant disease will be assessed. This new technology fully retains the antimicrobial capacity of the bacteriophages and enhances its stability, particularly against dehydration, making this technology a potentially good candidate for use of biocontrol agents for crop pathogen treatment.

S17/P53

Using Synthetic Frog Foam as a Novel Drug Delivery System for Topical Antibiotic Treatment.

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Engystomops pustulosus is a frog species found in Trinidad. They lay their eggs in highly stable foam nests which consist of six proteins called Ranaspumins (RSNs). RSN-2 is the main surfactant protein which is non-toxic to mammalian cells. An interesting feature of the foam is its ability to uptake dyes or drugs, which are then released over time. These properties indicate the foam could be used as a potentially novel drug delivery system, which could be developed for topical antibiotic treatment.

A dynamic dialysis method was applied to test the capability of *E. pustulosus* foam to releasevancomycin, calcein and Nile red. These demonstrated a stable release over three days, and up to one week. Initial rheology experiments indicated that the foam could withstand 90 Pa of pressure. RSN-2 and RSN-3 were overexpressed in *Escherichia coli.* Expressed RSN-2 demonstrates surfactant activity, forming a short lived foam, indicating that the other RSNs aid foam stability.

Future works include performing toxicology experiments using HeLa epithelial cells, and investigating the release of antimicrobial agents such as Silver Sulfadiazine. Further, the killing ability of foam released vancomycin will be tested against *Staphylococcus aureus* and *Pseudomonoas spp*. The final goal is to use *E. coli* expression constructs to produce a synthetic foam, which could be utilised as a drug delivery system. As skin infection remains a major problem in burn treatment, the aim is to construct a local foam antibiotic delivery system which could reduce the need for IV antibiotics in burn patients.

S17/P54

Effluent treatment and reuse through application of coliphages and natural reed bed: A potential strategy for safe use of wastewater in DRDO biotoilet technology

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Poor sanitation is a burden to society. Developing eco-friendly, appropriate and affordable sanitation solution is essential in flush toilet system. Water is the integral part of this globally

familiar system, including India. This work intended to develop minimizing sludge generation (to reduce human intervention) and effluent water reutilization (for re-flush or other purposes) to reduce indiscriminate use of fresh water for flush.

As an important contributor to 'Clean India Mission', DRDO Biotoilet is an appropriate technology comprising an anaerobic digester tank (Biotank) and a reed bed. After bacterial digestion, effluent water flows through reed bed for further cleaning and reuse (such as flushing etc). Pathogenic bacteria of the effluent water can be treated biologically using bacteriophages present in the system. To cater the need of the system, a group of natural bacteriophages present in the effluent has been enriched in the laboratory. Emphasis was given to enterobacteriacae group, especially for coliphages. Classical methods using plaque assay, electron microscopy and metagenomic techniques have been carried out to develop efficient phage consortium. Temperature and phage yield relationship study was also carried out to standardize the conditions required for enrichment. Metagenomic study revealed varied group of phages (podoviridae> siphoviridae> myoviridae). Laboratory experimentation and field studies have proven the efficiency of enriched phage consortium along with reed bed to reduce the coliforms from the effluent water. This water is safe to supply to toilet-flush that can help in fresh water conservation and reduce water pollution.

S17/P55

The bee orchid (*Ophrys apifera*) and its relationship with fungi

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Mycorrhiza is a crucial symbiotic association between the roots of plants and fungi within the soil environment. The specificity in these partnerships, particularly of the fungal partner, is one area where there are large gaps in knowledge. Orchids require symbiotic fungi in a mycorrhizal association for seed germination and establishment, typically species within the Tulasnellaceae and Ceratobasidiaceae. The distribution of suitable mycorrhizal fungi will therefore affect the distribution of orchid plants. The bee orchid (*Ophrys apifera*) is considered common in southern England but is less frequent in the north. It has a conspicuous flower spike with individual flowers resembling bees. It requires nutrient poor, calcareous soils. The species is surprisingly common in urban waste and reclaimed ground where rubble from buildings provides suitable well-drained, alkaline and low nutrient conditions. The rosette of leaves grows tightly against the ground so has some protection from mowing. We are using molecular ecology methods and morphological characteristics to help us understand the relationships between fungi and urban bee orchids. In addition, physiological relationships between the plant and fungus can involve comparisons between orchids in aseptic culture and in the ground.

S17/P56

Microarray-based analysis of pathways for degradation of hydrocarbons in oil-polluted marine environmental samples.

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Naturally occurring oil-degrading microorganisms play a major role in the processes of oil biodegradation in the sea, and it is important to assess their biodegradation capacity in polluted systems to rationally design the interventions, such as bioaugmentation or biostimulation. One of the methods to achieve that is the use of microarray-based genomic technologies that represent a powerful tool for analysis of a metabolic potential of microbial community in environmental samples.

The purpose of this work was to reconstruct the networks of biodegradation pathways through the implementation a previously designed microarray chip (Vilchez-Vargas et al., 2013), which is based on the set of genes with experimentally validated functions. The microarray was applied to evaluate the complex microbial communities from the environmental samples from chronically and accidentally polluted marine environments, namely, samples from Petrochemical Plant in Gela (Sicily, Italy) and with oil-spiked enrichment cultures set up from the same site. Overall, crude oil pollution increased the total number and diversity of catabolic genes as compared to control pristine samples. Moreover, different treatments (with and without dispersant) showed differences in gene content that were detected by microarray in our experiments. The results obtained so far indicate that microarray-based analysis can be an efficient tool for screening of large set of genes associated with hydrocarbon degradation in oil-polluted marine environmental samples.

S17/P59

Prediction and discovery of complete ammonia oxidation (comammox)

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Since Winogradsky's discovery of nitrifying bacteria in 1890, it was known that ammonia oxidation occurs in two steps: (1) incomplete oxidation of ammonia to nitrite by ammonia oxidizing bacteria, or as recently discovered, archaea; (2) oxidation of nitrite to nitrate by nitrite oxidizing bacteria. We have argued in 2006 that this metabolic division of labour can be explained by kinetic theory of optimal pathway length, predicting that the shorter metabolic pathway of incomplete ammonia oxidation can sustain a higher rate of ATP production and therefore a higher rate of growth (Costa et al. 2006). However, we also argued that complete ammonia oxidation to nitrate by a single organism would have the advantage of a higher growth efficiency or yield, and that this higher yield would make this organism more competitive when growing in biofilms. Therefore, we predicted the existence of this organism we called "comammox" for complete ammonia oxidizer in honour of "anammox", the anaerobic ammonia oxidizers discovered by Kuenen's group at the TU Delft in 1995. González-Cabaleiro et al. (2015) argued that comammox would be outcompeted in the end. Recently, two Nature papers published back to back reported the discovery of comammox in a fish tank in Radboud University's animal unit (van Kessel et al. 2015) and in an oil well exploration pipeline 1,200 m below ground in Russia (Daims et al. 2015). We discuss the extent to which these two discoveries validate our predictions and the underlying kinetic theory, which we suggest also applies to other food chains.

S17/P60

Investigations into the extremophilic properties of Exiguobacterium sp strain – HUD by genotypic and phenotypic methods

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Aims

Species from the genus *Exiguobacterium* have been identified in extremes of pH and isolated from environments of extreme temperature, including permafrost and hydrothermal vents (1). The aim of this study was to investigate the genotypic and phenotypic characteristics of a novel *Exiguobacterium* in terms of pH, temperature and heavy metal stresses.

Methods and results

The isolate was obtained from mesophilic sediments that had been gradually attenuated to an alkaline pH up to pH10.0 (2). A whole genome sequence was obtained for the isolate through shotgun sequencing (3). Subsequent annotation indicated the presence of stress response genes associated with extremes of pH, temperature and presence of heavy metals. Phenotypic characterisation showed that the isolate was tolerant of pH values between 4 and 12 and temperatures of 25-50°C.

Conclusions

Despite being obtained from a mesophilic sediment, the genome of the isolated *Exiguobacterium sp* indicated that the genes associated with survival in extreme conditions were retained. Utilisation of these genes was observed through survival or growth at extremes of pH and temperature. This broad spectrum of activity suggests that the potential for heavy metal resistance and MIC values have been obtained.

Significance of study

The genotypic and phenotypic characterisation of this novel *Exiguobacterium* allows for its potential use for industrial purposes. The strain may be selected for the generation and isolation of potentially novel thermo- or alkali-stable enzymes. Potential resistance to pollutants such has heavy metals may suggest that the strain could be used for targeted bioremediation via broad substrate utilisation.

S17/P62

Investigating the rumen metavirome.

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In recent years rumen research has focused on the role of bacterial and archaeal species on rumen function. Due to this the rumen metavirome is an understudied area, with only 2 previous studies having looked at the diversity and function of viruses in the rumen. This means that the role of viruses in horizontal gene transfer (HGT) and population control have been overlooked as well as looking at the diversity of species of viruses and their relative abundances.

A virome assembly was produced by combining all previous rumen metavirome studies, providing over 30,000 viral confirmed contigs. Next a dataset of 1.5Tb of metagenomic sequence data was aligning to the assembly, extracting viral reads (300 million reads). Using these reads we were able to estimate abundance and diversity within the metavirome. As this method relied heavily on bioinformatic techniques for extraction of viral sequences, multiple tests were conducted to ensure our metavirome was truely viral.

SNPs were called from the virome to study the diversity of quasi-species within the rumen as well as understand which viruses are adapted for their role within the rumen and which are constantly having to adapt.

A metagenome was also produced from the 1.5Tb dataset, allowing us to select CRISPR arrays and produce a network of viral/host interactions.

S17/P63

The involvement of the recA-hydN genomic region in Salmonella enterica Thyphimurium during the perception of nitric oxide

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Biofilms formed in post-harvest production facilities are reservoirs of pathogens, which are resistant to common disinfectants. Recent research showed that nitric oxide donors could be used to disperse biofilms. The capability of nitric oxide donors to disperse biofilm formed by human pathogens and genes involved in this process was investigated. The nitric oxide donor MAHMA NONOate is able to disperse biofilm formed by Salmonella enterica Thyphimurium . Salmonella biofilms were pre-formed on polystyrene and exposed to 1µM MAHMA NONOate. Biofilm dispersal was measured by staining the biofilms using crystal violet and measured with a spectrophotometer. MAHMA NONOate was able to disperse the biomass up to 50% when compared with the not treated. In order to identify the genes involved in this process, a deletion mutant and qPCRs were performed to determine the genes involved in the metabolic cascade downstream the perception of nitric oxide in Salmonella. We identified the genomic region recA-hydN leading to an insensitivity to the nitric oxide donors. This region includes putative NO-reductase machinery: ygaA, an anaerobic nitric oxide reductase transcriptional regulator; STM2840, an anaerobic nitric oxide reductase flavorubredoxin; ygbD, nitric oxide reductase; hydN, an electron transport protein HydN. Proteins encoded region can be involved in transferring electron to NO, its detoxification and generation of nitrous oxide. In addition, by using qPCR we identified that the gene mltB, a putative membrane-bound lytic transglycosylase, is repressed upon exposure to 1mM MAHMA NONOate within 40 minutes of exposure.

S17/P64

Bacterial contamination of cat food left in a household environment.

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Increasing pet ownership and the increase in antibiotic resistant microorganisms poses a significant threat to animal health. In addition, Salmonella, and other bacterial contaminants, have been shown to cause illness in humans as a result of contact with contaminated dog food. Research into the possible contamination of dry cat food has been very limited even though many studies have reported contamination of dry dog food. It may be possible that contamination occurs during the manufacturing process but it is also likely that contamination occurs upon exposure to the animal. Food and water samples were collected after 24 hours of cat exposure, and then stored at room temperature in order to mimic environmental variables. Survey results from owners were used to determine how long pet food and water is exposed to potential contamination in the home environment and microbiological analyses determined the contamination levels of the food and water over time. Microorganisms were cultured from food and water samples using standard techniques. Each type of bacteria found, presumptively Pseudomonas spp. and Staphylococcus spp., were identified. The public and animal health concerns over leaving food and water out for long period of time will be considered.

S17/P68

Antimicrobial susceptibility profile of *Plesiomonas shigelloides* recovered from some rivers in Southwestern Nigeria: implications for public health

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We investigated the antimicrobial susceptibility profile of *Plesiomonas shigelloides* isolated from some rivers in Osun and Oyo States, Southwestern Nigeria. Water samples were collected over a period of 4 months (May to August, 2015) and analysed using standard procedures. A total of 150 presumptive isolates were recovered and their identities were established by polymerase chain reaction (PCR) technique using PS gene marker. Thirty eight (25%) of the isolates were confirmed as *Plesiomonas shigelloides* specie and these were screened for *in vitro* antimicrobial susceptibility profiles using disc diffusion assay. Susceptibilities to antibiotics are as follows; meropenem (100%), imipenem (97%), gentamycin (94%), enrofloxacin (63%), amikacin (61%), ciprofloxacin (58%), and ceftazidine (55%). Conversely, all the isolates were resistant to sulphamethoxazole and erythromycin, and variously resistant to other antibiotics as follows; ampicilin (97%), cefuroxine (97%), neomycin (84%), chloramphenicol (74%) and cefotaxine (68%). Conclusively, our findings reveal the presence of *Plesiomonas shigelloides* in the selected rivers and also signify high resistances towards the conventionally used antibiotics, and consequently jeopardizing the usefulness of the waters for domestic, industrial and recreational purposes.

S17/P69

Use of a dual gene reporter system to monitor *Listeria monocytogenes* attachment to solvents

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Listeria monocytogenes causes listeriosis in humans and animals and contaminates prepared food by attaching to food processing environments. Therefore, a closer monitoring of how the organism fixes itself on surfaces will help in finding ways to prevent the organism from colonizing food processing environments. To develop new attachment assays, clinical and environmental strains of *L. monocytogenes* were transformed by inserting a plasmid containing *lux*, *gfp* reporter genes and selective erythromycin resistance genes into the parent cells. Transformed cells were grown for 48 hours on Brain Heart Infusion agar plates containing 5µg/ml of erythromycin after which the cells were viewed under a molecular light imager. Fluorescent cells containing the *gfp*, *lux* and erythromycin resistant genes were visible whereas control cells without the plasmid were not visible under the imager. Transformation efficiency was highest with the environmental strains and subsequent growth and hydrophobicity tests carried out with the transformed cells in different growth conditions showed that they were able to attach well on solvents when compared to the parent cells. Results indicate the possibility of real time monitoring of how the cells attach on solvents and could lead to a better understanding of the initial colonisation of surfaces by the organism.

S18/P1

Avian IFITMs: Restricting Non-enveloped Virus Replication

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Interferon inducible transmembrane (IFITM) proteins are located in the plasma and endosomal membranes, and are known to restrict the replication of viruses in host-cells. Studies in mammalian cells suggest they inhibit the entry of both enveloped and non-enveloped viruses, however surprisingly little is known about the antiviral function of avian IFITM proteins. In order to address this, we characterised the effect of chicken IFITM (chIFITM) proteins on the replication of the non-enveloped avian birnavirus, infectious bursal disease virus (IBDV), *in vitro*. The expression of chIFITM 1, 2, 3 and 5 mRNA in avian DF-1 cells significantly increased following infection with IBDV, strain D78 by 51, 21, 5 and 53-fold respectively ($p < 0.05$). Moreover, in DF-1 cells that were pre-treated with siRNA constructs to knock-down chIFITM 1, 2 and 3 expression, the replication of D78 increased by an average of 2.3, 3.8 and 1.9-fold respectively, compared to cells treated with a scrambled siRNA control. This reached statistical significance for chIFITM 1 and 2 ($p=0.01$), but not for chIFITM 3 ($p = 0.07$). In DF-1 cells transiently overexpressing chIFITM proteins 2, 3 and 5, the average replication of D78 was reduced by 0. 3, 0.6 and 0.4-fold respectively, compared to cells that were transfected with a negative control, however these data did not reach statistical significance. Taken together, these data suggest that chicken IFITM proteins restrict the replication of non-enveloped IBDV strain D78 in avian DF-1 cells, and ongoing work is aimed at confirming this hypothesis.

S18/P2

Role of PI3K a host cellular signalling pathway in innate immune response to influenza virus infection in chicken cells

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Highly pathogenic avian influenza viruses (HPAIVs) arise during replication of certain low pathogenicity influenza A virus (LPAIV) subtypes in poultry flocks. LPAIVs tend to efficiently replicate in chickens but cause either mild or no disease. In contrast, when HPAIVs emerge in chickens, they cause high mortality. Understanding host cellular responses to influenza virus infections in chickens is of key importance to develop better disease control strategies. Increasing evidences suggest that influenza virus infection modulates diverse host cellular signalling pathways including the phosphatidylinositol 3-kinase (PI3K/Akt) signalling pathway. It is well established that the PI3K/Akt pathway is responsible for cellular growth, proliferation, differentiation, survival and inhibition of pro-apoptotic factors. We investigated the role of the PI3K/Akt pathway in virus replication and host cellular response in primary chicken embryo fibroblast (CEF) cells. Cells were infected with LPAI virus strains (subtypes H2N3 and H6N1) or three 6:2 recombinant viruses generated using a PR8 reverse genetics system with 6 internal genes from HPAI H5N1 strains (50-92 or Tky05) or H9N2. The PI3K pathway was activated in the later phases of influenza virus replication in chicken cells and chemical inhibition of the PI3K pathway significantly reduced virus output. Interestingly, the PI3K pathway was downregulated at 9 hours after infection with the LPAI viruses but was still activated at 24 hour after infection with the HPAI H5N1 reassortant viruses. This raises the potential role of PI3K signalling pathway in contributing towards the efficient replication of influenza virus and disease severity in chickens and warrants further studies.

S18/P3

ANTIVIRAL ACTIVITY OF PRIMATE FICOLINS AGAINST HEPATITIS C VIRUS (HCV) AND OTHER EMERGING VIRAL INFECTIONS

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Hepatitis C virus (HCV) causes chronic liver diseases in 3% of the world's human population. HCV encodes two envelope glycoproteins, E1 and E2, which are found on the surface of the viral lipid envelope. These glycoproteins facilitate entry into the host. They have been shown to possess epitopes that are targets for host immune recognition.

Ficolins are liver-expressed pattern recognition receptors that contribute to the innate surveillance of virus infections, recognising carbohydrates such as N-acetyglucosamine. Three ficolin genes have been identified in humans; FCN1, FCN2 and FCN3. These encode the proteins M-ficolin, L - ficolin and H-ficolin, respectively. L-ficolin has been demonstrated to bind to HCV virions and inhibit infection. However, HCV persists in the presence of this protein, suggesting that the virus may adapt to avoid recognition by host immune effector.

Three ficolin-encoding genes have been identified in all non-human primates, while two are found in mice (fcn-a and fcn- b). However, the antiviral activity of non-human ficolins is unknown. Thus we sought to assess the anti-viral properties of these pattern recognition receptors from different mammalian species. Ficolin genes were obtained from gorilla, marmoset and colobus primate species, and mice. These proteins were cloned, expressed and purified by affinity chromatography. Initial experiments assessed the ability of these proteins to inhibit HCV

infection in an established entry model. Future work will examine the breadth of reactivity of these proteins against a range of viruses, including zoonotic infections such as Ebola and rabies viruses.

S18/P4

Modulation of interferon induction by the murine norovirus virulence factor 1 (VF1) protein.

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Human norovirus (HuNoV) is one of the commonest causes of acute gastroenteritis worldwide. There is currently no approved treatment for HuNoV infection, and efforts at designing potent therapeutics are hampered by our limited knowledge of norovirus pathogenesis and lifecycle, owing to lack of a robust cell culture system. Murine Norovirus (MNV) has been used for over a decade as a surrogate model for studying the biology of noroviruses, as the only member of the Norovirus genus with a robust cell culture system. Depending on the strain of MNV used and the genetic background of the host, MNV can cause acute, lethal or persistence infections in its natural host, namely mice. Therefore, in addition to providing a tool for the study of norovirus biology, MNV provides a robust experimental system to understand viral pathogenesis as well as the contribution of viral and host factors to viral persistence.

The MNV VF1 protein was recently shown to antagonize antiviral innate immune responses, where cells infected with a VF1-deleted virus showed higher induction of interferon-beta and interferon-stimulated genes. VF1-deficient mutants reverted to the wild-type virus following passage in cell culture, suggesting a fitness cost to the virus. Following infection, VF1 localizes to the host mitochondria, suggesting a potential interaction with mitochondria-associated innate immune signalling complexes. However, the exact mechanism through which VF1 modulates innate immune responses is not known. In this study, we use in vitro infection assays and transient over-expression in cell lines to interrogate the intracellular topology and mechanism of VF1 immunomodulation.

S18/P5

Characterisation and identification of chicken PML.

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Promyelocytic leukemia protein (PML) is the major component of nuclear multi-protein complexes known as PML nuclear bodies (PNB). PNBs are involved in various cellular activities ranging from growth suppression, apoptosis and anti-viral. PNB proteins and their functions in mammalian cells are studied widely. However, there is a lack of knowledge in the presence and function of PNB proteins in other vertebrates. In this study we have identified two genes in chicken that produce proteins with a nuclear distribution similar to that of mammalian PML: PML and PML-Like (PML-L). PML and PML-L proteins share over 70% identity with each other and about 30% identity with their human counterpart. Here in this study, in an attempt to identify the interacting proteins with PML in PNBs, we have aimed to isolate the PNB complexes by immunopercipitation and have identified the interactome of the proteins be employing mass spectrophotometry techniques. To study the effect of the proteins on virus replication, we have produced recombinant cells from avian origin which constitutively express PML proteins. We have also measured the replication of avian influenza virus H9N2 using the cells to study the antiviral effects of the proteins.

S18/P6

Relative cytopathology in RSV-infected well-differentiated paediatric primary nasal epithelial cell cultures derived from cohorts of infant with histories of severe or mild disease.

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One to 3% of infants are hospitalised with bronchiolitis or pneumonia due to RSV annually. The vast majority of infants, therefore, do not suffer severe disease. Airway epithelial cells are the principal targets for RSV infection in humans, while RSV load in airway secretions correlated with disease severity. We hypothesised, therefore, that RSV infection would result in greater cytopathogenicity, virus growth kinetics, and pro-inflammatory chemokine responses in airway epithelium from

infants with histories of severe RSV disease versus mild disease. To address this, we exploited RSV-infected well-differentiated paediatric primary nasal epithelial cell cultures (WD-PNECs). The epithelial cells were derived from cohorts of infants with clinical histories of severe or mild RSV disease. WD-PNECS from each cohort were indistinguishable in terms of ciliated and goblet cell content and tight junction integrity. RSV BT2a (clinical isolate) infection of WD-PNECs was restricted to apical ciliated cells but caused no noticeable damage to cultures from either cohort. Virus growth kinetics and syncytia formation were also similar in both. Interestingly, apical cell sloughing and apoptosis was slightly increased in RSV-infected WD-PNECs from mild, but not severe, cohorts relative to uninfected controls. However, pro-inflammatory responses did not differ following infection, as measured by RANTES, IP-10, TRAIL and MMP secretions, although IL-29 secretions were diminished in the severe cohort. Our data suggest that there are few substantial differences in RSV cytopathogenesis, replication and pro-inflammatory responses in WD-PNECs derived from severe and mild cohorts that might explain the differential disease severity between them.

S18/P7

Elucidation of the role of an innate antiviral protein in respiratory syncytial virus infection.

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Respiratory syncytial virus (RSV) causes severe disease in 1-3% of infant cohorts annually. Reasons why some infants become seriously ill, while the majority cope reasonably well with RSV infection remain to be elucidated. A microarray study was undertaken comparing transcriptome responses in well-differentiated paediatric primary nasal epithelial cell cultures (WD-PNECs) derived from cohorts of infants with histories of mild or severe RSV disease. Gene x was identified as downregulated at baseline in children with a history of severe disease compare to mild disease and was unaffected by RSV infection. Recombinant protein X reduced RSV infection in both cell lines and well-differentiated primary paediatric bronchial epithelial cells (WD-PBECs) following pre-treatment at 4oC. Pre-treatment of BEAS-2B cells with a neutralising antibody targeting X resulted in significant increases in RSV infected cells. To determine whether protein X affected RSV entry, BEAS-2B cells incubated on ice and infected with RSV followed by treatment with protein X. Following shifting to 37oC for 10 min, the cells were washed with citrate buffer (pH 3.0), rinsed with PBS, fresh medium added and incubated at 37oC. Under these conditions, a reduced number of RSV-infected cells was evident in the protein X-treated cultures compared to controls, indicating that protein X attenuates RSV entry to BEAS-2B cells. Our data suggest that protein X acts as an innate antiviral molecule in respiratory epithelium by interfering with binding and/or entry of RSV to the cells and that the relative endogenous quantity in infant airways contributes to susceptibility to severe RSV disease.

S18/P8

Well-differentiated primary nasal epithelial cell (WD-PNEC) cultures derived from newborn term, preterm and cystic fibrosis infants: an exciting opportunity to study airway innate immune responses to respiratory syncytial virus infection in high risk groups.

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Respiratory syncytial virus (RSV) is the commonest cause of severe lower respiratory tract infection in infants under two-years worldwide. Young and premature infants and those with respiratory disorders, such as, cystic fibrosis are at greater risk of severe RSV-related disease. Little is known about the airway epithelial responses to pathogens in these groups. We aimed to establish and characterise WD-PNEC cultures derived from term and preterm infants and cystic fibrosis infants, within hours/days of birth and/or diagnosis, in terms of morphology and responses to RSV infection.

Interdental brushes were used to obtain nasal epithelial cells from term and preterm infants within 48 h of birth. Infants with cystic fibrosis were recruited at the time of diagnosis on newborn screening (6-8 weeks old). Morphologically and physiologically authentic WD-PNECs were successfully generated from the cells, as characterised using light microscopy and immunocytochemistry. Newborn WD-PNEC cultures had extensive cilia coverage and mucous production, while transepithelial electrical resistances were invariably >500 Ω.cm2. Robust RSV growth kinetics were evident following infection, although little cytopathogenesis was evident following infection of newborn or CF infant-derived WD-PNECs. These newborn WD-PNECs represent a unique opportunity to study differential airway epithelium innate immune responses to RSV in very early in life.

S18/P9

Elevated H- and L- ficolin expression is associated with hepatocellular carcinoma in hepatitis C virus infections

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Chronic Hepatitis C virus (HCV) infection can lead to hepatocellular carcinoma (HCC). The innate immune pattern recognition receptors (PRRs) H- and L-ficolin, and the Mannose Binding Lectin (MBL) neutralize virus entry. This may contribute to controlling HCV infection. However, the age and sex of an infected individual may affect the expression of these proteins. We aimed to investigate these factors in a cohort of HCV-infected individuals, to determine the association of PRR concentrations with disease progression. This approach was proposed by analyzing serum samples from 1518 well-characterized patients with mild infection, 160 with HCC, and 144 HCV-negative controls. MBL, H-ficolin, and L-ficolin were assayed by ELISA. Significant differences existed between the level of both ficolins in males and females, expression declining with age. In contrast, MBL expression was maintained in all age groups. In HCV-infected individuals notably high levels of H- and L-ficolin were detected early in infection, and elevated levels were observed in samples taken before the diagnosis of HCC. The expression of H-ficolin was lower in age-matched non-severe HCV infections. Low levels of MBL were detected in all age groups early in infection. The level of ficolins clearly increased with the progression of HCV-induced disease, particularly in HCC cohorts. As such this protein could be used as a serum biomarker of disease progression.

S19/P1

Toxigenic Clostridium difficile isolates from clinically significant diarrheal patients in a tertiary care center

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Introduction: *Clostridium difficile* is the primary cause of hospital acquired colitis in patients receiving antibiotics. The pathogenicity of the organism is mainly due to production of toxins.

Objectives: We performed toxigenic culture of fecal samples from patients suspected of *C. difficile* infection (CDI) and analyzed their clinical and demographic data.

Materials and Methods: Details of 1110 diarrheic patients included in the study were recorded in a pre-printed proforma and analyzed. Fecal samples obtained from them were cultured for *C. difficile* and the isolates identified by phenotypic and molecular investigations. *C. difficile* from broth culture was identified for toxigenicity using enzyme linked immunosorbent assay for toxins A and B.

Results: Of the 1110 cases, 63.9% were males. The mean age of the patients was 39 years. The major antibiotics received by the patients were nitazoxanide (23.9%), penicillins/penicillin combinations (19.0%), quinolones including fluroquinolones (13.1%), carbapenems (11.5%), glycopeptides (11.0%) and cephalosporins (8.4%). Predominant clinical symptoms present were watery diarrhea (56.4%), abdominal pain (35.3%) and fever (40.0%). The underlying diseases were gastrointestinal disorders (52.6%), cancers (13.2%), surgical conditions (8.3%), hepatic disorders (8.0%), blood disorders (4.5%), renal disorders (3.6%), respiratory disorders (3.2%), neurological disorders (2.4%), tuberculosis (2.0%), cardiac disorders (1.3%) and skin infections (0.8%). Of 174 *C. difficile* isolated 54.6% were toxigenic. Toxigenic *C. difficile* was present in all (100%) patients with surgical conditions followed by patients with cancer (65.2%) and gastrointestinal disorders (57.1%).

Conclusions: Clinical conditions of the patients correlating with toxigenic culture can be a valuable asset for diagnosis of CDI.

S19/P2

Occlusion of stents of patients with biliary and pancreatic diseases by various bacterial community

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Introduction: Bacterial biofilms are matrix-enclosed bacterial population adherent to each other and to surfaces. They have an enormous impact on medicine as they can form on many implants such as stents and catheters. Biofilm infections are rarely resolved by the immune system of the host.

Objectives: In the present study, we characterized the diverse bacterial community in biofilms of the stents retrieved from patients with biliary and pancreatic disease.

Materials and methods: A total of 105 stents from patients with biliary diseases were processed for investigation of bacteria occluding the biofilms. Commonly known bacteria were identified in the biofilms by PCR. The amplified PCR products were sequenced using selected bands excised from gel to identify the unknown bacteria.

Results: The commonly known bacteria identified were *Pseudomonas* (n=34), *Escherichia coli* (n=17), *Citrobacter* (n=23), *Streptococcus* (n=17), *Aeromonas* (n=17), *Clostridium* (n=1), *Enterococcus* (n=13), *Staphylococcus* (n=25), *Proteus* (n=14), *Bacillus* (n=6), *Klebsiella* (n=24), *Enterobacter* (n=12), *Serratia* (n=29), *Yersinia* (n=7) and *Vibrio* (n=2). The uncommon bacteria found responsible for occluding a large number of biliary stents were several *Pseudomonas* species (n=17), several *Bacillus* species (n=14), *Stenotrophomonas maltophila* (n=13), *Enterococcus faecalis* (n=6), *Enterococcus durans* (n=3), *Enterococcus* species (n=1), *Enterobacteriales bacterium* (n=9), *Citrobacter freundii* (n=2), *Citrobacter sp.* (n=2), *Staphylococcus epidermidis* (n=3), *Klebsiella* (n=3), *Chryseobacterium sp.* (n=3), *Micrococcus yunnanensis* (n=1), *Micrococcus luteus* (n=1), *Morganella morganii* (n=2), *E. coli* (n=1), *Vibrio cholerae* (n=1).

Conclusion: There was a high collection of biofilms in the biliary stents of the patients with rich bacterial community of commonly known and uncommon organisms.

S19/P3

Diagnosing of mixed respiratory bacterial infections by multiplex PCR

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Atypical bacteria grow very slowly in culture or they do not grow at all leading to delays in detection and diagnosis. PCR multiplex was performed on template DNAs extracted from seventy three collected specimens. Thirty seven showed positive indication for the presence of bacterial infection. The incidence of *Mycoplasma pneumoniae*, *Chlamydia pneumonia* and *Legionella pneumophila* as a single infecting agent was 31.5%, 27.5% and 20 % respectively. Dual agent infection caused by *Mycoplasma* + *Chlamydia*, *Mycoplasma* + *Legionella* and *Legionella* + *Chlamydia* was 24%, 20% and 15% respectively. Triple agent infection caused by *Legionella* + *Mycoplasma* + *Chlamydia* was 17.5%. The etiology of the infection was *M. pneumoniae*, *L. pneumophila* or *C. pneumoniae* as a single etiology or in combination of two or three organisms.

S19/P4

DROP OF POST-OPERATIVE INFECTION AFTER PRE-OPERATIVE VACCINATION WITH ANAEROBIC TOXOIDS (A CLINICAL STUDY OF 445 PATIENTS)

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Background:

After a successful experiment in rats with anaerobic vaccine in combating peritonitis in the model of ceacal ligation and puncture to prevent sudden death, it was decided to undergo a clinical study.

Material and methods:

445 patients who underwent major and semimajor operations were vaccinated each with 0.25 mL of covexin 10 before operation.

Results:

Infection of the wounds occurred in 24 of 445 patients (5.4 %). Infection occurred in 7 of 202 who underwent breast operations, mostly for breast cancer including axillary dissection (3.5 %). Infection occurred in 2 of 50 who underwent major operation for colorectal cancer (4%). 3 of 43 patients who underwent major operation for urinary bladder cancer developed wound infection (7.0%). Other operations for other sites were associated with a low incidence of wound infection compared to historical control.

Although, it was a large series of major operations, not a single case developed multiple organ failure syndrome.

Discussion:

Because of success of anaerobic vaccination in combating post-operative wound infection, we recommend that researchers in this field to start similar clinical trials. Because multiple organ dysfunction syndrome was not encountered in this series, a possible etiology of the syndrome may be due to toxins released from anaerobic sepsis in small bowl.

Conclusion:

Vaccination with anaerobic vaccine was successful in reducing post-operative wound infection to about half of historical control.

Key words: wound infection, anaerobic vaccine, and anaerobic infection.

S19/P5

The potential of medieval 'ancientbiotics' in the treatment of chronic biofilm infection.

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We previously reported the (re)discovery of a 1,000-year-old remedy for soft tissue infection with exceptionally high bactericidal activity against *Staphylococcus aureus*, a clinically important coloniser of chronic wounds (*mBio* 6:e01129). We now present results from our testing pipeline demonstrating (a) quantitative data mining of pre-modern medical texts for novel antibacterial cocktails and (b) the use of laboratory models for testing the *in vitro* and *in vivo* potential of pre-modern remedies for treating recalcitrant infection. Specifically, we show that our first "ancientbiotic" (1) has a wide spectrum of antibacterial activity, killing clinical isolates of Gram-positive and Gram-negative species, including highly antibiotic resistant clinical isolates; (2) can kill pathogenic bacteria in polymicrobial biofilms grown in an *in vitro* chronic wound model; and (3) does not cause significant damage to human keratinocytes or trigger a pro-inflammatory immune response. We also present preliminary data suggesting that *S. aureus* cannot easily evolve resistance to this remedy. Our results illustrate the potential power of the ancientbiotic pipeline for discovering novel therapeutics to treat chronic biofilm infections, such as those found in burns, surgical sites, medical devices, pressure sores and diabetic ulcers.

19/P6

Virulence Potential Of *Klebsiella pneumoniae* Isolated From Neonatal Nasogastric Feeding Tubes

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The rate of neonatal infections due to *Enterobacteriaceae* has increased in neonatal intensive care units, particularly those born with low birth weight (< 2000g) and fed via nasogastric tubes. Moreover there has been limited consideration that the nasogastric enteral feeding tube (NEFT) may act as a site for bacterial colonisation and a locus for infection. The objective of the present study is to evaluate the potential virulence of *Klebsiella pneumoniae* isolated from neonatal NEFT from two Jordanian hospitals. In this study, 76 *K. pneumoniae* strains were isolated from neonatal feeding tubes, from May to Dec 2011. The isolates were identified by sequence analysis of the *rpoB* gene and genotyped using pulsed-field gel electrophoresis. Isolates were examined for potential, virulence factors, biofilm production, and antibiotic resistance. The strains clustered into five pulsotypes. The curli fimbriae and hypermucoviscous phenotype were observed in 3.1% and 12.9% of isolates respectively. Capsular serotypes included K1 (3.8%) and K2 (16.72%). All isolates showed resistance to imipenem, meropenim and ceftriaxone. An extended spectrum beta-lactamase was identified phenotypically in strains with resistance to cefotaxime + clavulanate and cefpodoxime + clavulanate, and most isolates showed susceptibility to ciprofloxacin. All strains were able to form biofilms on plastic surfaces at 25 oC and 37 oC.

K. pneumoniae strains exhibited features that would allow them to survive on feeding tubes and resist antimicrobial therapy. The bacterial colonisation of neonatal enteral feeding tubes may be one reservoir source which could constitute a serious health risk to neonates.

S19/P7

Characterisation and virulence factors of *Acinetobacter baumannii* strains isolated from neonatal feeding tubes in neonatal intensive care units.

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Acinetobacter baumannii, a major nosocomial pathogen with a particular ability to develop antimicrobial resistance and cause nosocomial infection is a considerable risk in neonatal intensive care units. The aim of this study is to determine the resistance to antibiotics and the presence of virulence factors of *Acinetobacter baumannii* isolated from neonatal nasogastric feeding tubes.

Forty five clinical strains isolated from neonatal feeding tubes from two neonatal intensive care units. They were identified by sequence analysis of the *rpoB* allele (438 bp) and genotyped using pulsed-field gel electrophoresis using the *ApaI* restriction enzyme. The strains clustered into five pulsotypes and blaOXA-51-like genes were present in all of the isolates. Phenotypically antibiotic resistance testing was performed by the Kirby-Bauer method. Majority of the strains were resistant to all β-lactams of the antibiotics tested, but susceptible to ciprofloxacin. The ability to form a biofilm was measured by the 96-well microplate crystal violet method. All strains showed the ability of form significant biofilms at 37°C on milk agar compared with TSA (39%). Capsule production on milk agar and XLD-media were negative for all strains investigated whereas all bacteria to expressed curli fimbria according to colony morphology on Congo Red-agar. Fifty-six percent of isolates produced β-haemolysis on horse blood agar.

The isolates revealed a high of biofilm formation and antibiotic resistance, these features could allowed them to survive and act as a locus on enteral feeding tube and persistence in hospitals where neonates are at high risk of nosocomial infection in neonatal intensive care units.

S19/P8

Manuka honey improves antibiotic activity against *Staphylococcus pseudintermedius*

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Staphylococcus pseudintermedius is a common zoonotic pathogen, first reported in humans in 2006. The emergence of multidrug-resistant *S. pseudintermedius* in large numbers of animals could lead to an increase in the infection rate in humans.

Manuka honey has been shown to inhibit many pathogens including *S. aureus* and MRSA and has been used successfully in clinical practice. It has been established that manuka honey can act synergistically against *S. aureus* and *P. aeruginosa in vitro* when combined with appropriate antibiotics. The aim of this study was to determine the ability of manuka honey to inhibit *S. pseudintermedius* growth and biofilm formation both alone and in combination with antibiotics.

The susceptibility of clinical isolates of S. pseudintermedius to gentamicin, enrofloxacin, chloramphenicol, clindamycin, penicillin G, tetracycline and manuka honey was tested using adapted EUCAST methodology. When antibiotics were combined with honey (below the MIC) susceptibility was significantly increased when tested using both AST plates and microbroth dilution (checkerboard) techniques (FICI values were calculated).

In addition the above, combinations also reduced the ability of cultures to form biofilm when compared to the antibiotics alone. Fluorescent microscopy demonstrated that use of honey/antibiotic combinations also increased cell death within 24 and 48 hour biofilms when compared to antibiotics alone.

This study highlights the potential for manuka honey to be utilised in a veterinary setting, increasing the susceptibility of bacterial pathogens to antibiotics. With further *in vivo* testing this may offer an alternate therapy to those animals with infections that are not responding to conventional therapy.

S19/P9

In vitro immune modulation of *Salmonella enterica* serotype Pullorum

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Salmonella enterica infection is a global problem which affects a wide range of animals and human. The avian specific serotype *S.* Pullorum infection produces systemic disease followed by a carrier state in convalescence birds where small numbers of bacteria persist within macrophages in the spleen. In comparison with *S.* Enteritidis, *S.* Pullorum has been found to induce increased levels of IL-4 and lower levels of IFN-γ in the spleen of infected birds, which may switch the immunity from a pro-inflammatory to an anti-inflammatory response. Different immune responses between persistent *Salmonella* (*S.* Pullorum) and non-persistent (*S.* Enteritidis and *S.* Gallinarum) serotypes were compared ex vivo using Salmonella-infected macrophages and CD4+ T lymphocytes. In comparison with *S.* Enteritidis and *S.* Gallinarum, which induced an IFN-γ-producing Th17 response, macrophages infected with *S.* Pullorum had a reduced expression of IL-18 and IL-12α and stimulated proliferation of Th2 lymphocytes with reduced IFN-γ and increased IL-4. However, our data shows no evidence of clonal anergy or immune suppression induced by *S.* Pullorum. In conclusion, *S.* Pullorum modulates host immunity from a dominant IFN-γ-producing Th17 response towards a Th2 response which may promote the persistent infection in chickens.

S19/P10

The Effects of an Anglo-Saxon "ancientbiotic" on Bacterial Colonisation of Wounds

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We have previously shown that an Anglo-Saxon remedy for eye infection (Bald's eyesalve) has powerful antimicrobial action against *Staphyloccoccus aureus* (Harrison et al., 2015, *mBio*). While it has been established that Bald's Eye Salve is an effective at killing established *S. aureus* biofilms in *in vitro* wound models, an ability of eye salve to prevent various species of bacteria colonising wounds could be where its true potential lies. Prophylactic prescription of broad-spectrum antibiotics in patients with deep wounds as well as those who are post-surgery to prevent a nosocomial infection is a contributing factor in the emergence of antibiotic resistance (Enzler, Berbari, & Osmon, 2011). We show here initial evidence that pre-treatment of *in vitro* wound models with Bald's eyesalve prevents colonisation of wounds by *Staphylococcus* and other species. This may be provisional evidence that the eyesalve has potential to be used to prevent the infection of wounds in a clinical setting – perhaps as an adjuvant to wound dressings. This in turn could reduce the pervasive use of antibiotics.

S19/P11

Isolation and characterisation of four novel bacteriophages infecting clinically relevant strains of *Clostridium difficile*

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Clostridium difficile is a Gram positive, anaerobic, endospore-forming bacterium and one of the leading causes of hospital-acquired diarrhoea, causing a substantial financial burden on health systems. Routinely used treatments comprise the broad-spectrum antibiotics metronidazole and the 'last-line-of-defence' antibiotic vancomycin. Both cause collateral damage to the GI-microflora, the very thing which pre-disposes patients to *C. difficile* infection (CDI) in the first instance. There is clearly a need for alternative and more targeted therapies. One such possibility is bacteriophage therapy as the high specificity of phages (viruses which kill bacteria) will eliminate the damaging effect on the gut microbiota, likely leading to reduced incidence of relapse.

Four phages (phiCD08011, phiCD2301, phiCD418 and phiCD1801) which infect clinically relevant *C. difficile* ribotypes (002, 014, 023 and 078) have been isolated from the Nottinghamshire environment. The genomes of the four phages have been determined using Illumina MiSeq and they have been manually annotated. Lysogens of the phages have been created for long term storage. Phage morphology has been determined using transmission electron microscopy (TEM) suggesting they are Siphoviridiae. This was further confirmed with the identification of sipho-like tail proteins within the genomes.

Further characterisation of these phages is underway, including determining the host range of infection, burst size, adsorption rate and identifying phage receptors.

S19/P12

Novel Genetic Determinants of Intrinsic Antimicrobial Resistance in *Acinetobacter baumannii*

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Objective: The objective of this study is to discover novel genetic determinants of intrinsic antimicrobial resistance in *Acinetobacter baumannii*, a major nosocomial pathogen that has become increasingly multidrug-resistant.

Methods: TnAraOut transposon mutagenesis on *A. baumannii* S1, an antibiotic-susceptible clinical isolate from the Network for Antimicrobial Resistance Surveillance (Singapore) collection, was carried out using pNJ17 and the mutants were selected on LB agar containing kanamycin (30 mg/L) and ciprofloxacin (0.5 mg/L). Gene deletion mutants were constructed by allelic replacement of A1S_1989 and A1S_1990 in S1 and ATCC17978. Antimicrobial susceptibility was determined using broth microdilution. qRT-PCR was performed using KAPA SYBR® FAST Mix and the transcripts were normalized to16S rRNA using the 2^{-DDCt} method.

Results: Genome-wide transposon mutagenesis of *A. baumannii* S1 identified A1S_1989 and A1S_1990 that caused increased resistance to several antibiotics when disrupted. These genes encode small hypothetical proteins that are unique to *A. baumannii* and *A. nosocomialis* and are not found in *A. pittii.* Targeted deletion of A1S_1989 and A1S_1990 in *A. baumannii* S1 and ATCC17978 confirmed their involvement in intrinsic resistance to fluoroquinolones, meropenem, tetracycline, erythromycin and trimethoprim. qRT-PCR analyses of parental and mutant strains found blaOXA-51 expression to be significantly increased in the deletion mutants.

Conclusion: A1S_1989 and A1S_1990, which are unique to *A. baumannii* and *A. nosocomialis*, prevent overexpression of *bla*OXA-51 and the development of intrinsic resistance to a wide spectrum of antibiotics.

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S19/P13

Assessing the impact of antimicrobial treatment on two strains of *Pseudomonas aeruginosa* with differing antibiotic resistance

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Antibiotic resistance continues to be a growing problem and *Pseudomonas aeruginosa* is a common pathogen exhibiting resistance to a range of antimicrobial compounds. *P. aeruginosa* is the second most common cause of wound infection and persists in chronic wounds despite treatment, for years. Unsuccessful treatment can result in a situation that favours the growth of antibiotic resistant pathogens meaning that when infection recurs they predominate, further impairing the success of subsequent treatment.

This study assessed the growth patterns of two strains of *P. aeruginosa* (PA01 and PA14) in co-culture in the presence and absence of sub-lethal doses of antibiotics to determine which organism predominated. It was hypothesised that growth in the presence of antibiotic should favour PA14 which is resistant to a number of different antibiotics. It was found that in planktonic culture PA01 predominated in the absence of antibiotics but PA14 was most numerous in the presence of antibiotics. In a biofilm the numbers of each microorganism were approximately equal when cultured in the absence of antibiotics, but in the presence of sub-lethal doses of antibiotic over 90% of the population were PA14.

A PCR method was developed to rapidly identify PA14 in a mixed culture of *P. aeruginosa* based on a PA14 specific gene (*ladS*). It was possible to identify PA14 from mixed culture with varying concentrations of antibiotics with a detection level equating to nanomolar quantities of genomic material suggesting that molecular methods could be used to identify resistant bacteria in wound swabs containing *P. aeruginosa.*

S19/P14

Studying the antimicrobial efficacy of *Crocodylus siamensis* hemoglobin hydrolysate

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Antimicrobial proteins are a group of antimicrobial agents that are phylogenetically ancient components of innate host defense mechanisms. The biological activity of these agents demonstrates potency against a broad spectrum of microorganisms. This study aims to investigate antibacterial activity of *hemoglobin hydrolysate (HH) from* Crocodylus siamensis blood that have been differently hydrolyzed for 2, 4, 6 and 8 hours. Antibacterial activity of HH was investigated by Minimal Inhibition Concentration (MIC), fluorescent microscopy using both gram positive (*S.aureus*) and gram negative bacteria (*E.coli, K.pneumonia*e and *P.aeruginosa*).The effect of the HH on iron homeostasis was investigated using β-galactosidase activity using *E.coli ftnA* and *bfd* linked reporter strains. The results showed that HH 2, 4, 6 and 8 hours antibacterial activity was apparented for the concentrations of 20 mg/ml (MIC value) against *E.coli* and *S. aureus*. Furthermore, there was antibacterial activity at the concentrations of 10 mg/ml against *K.pneumoniae* and *P.aeruginosa*. Fluorescent microscopy (using Live/Dead stain) found that HH 2, 4, 6 and 8 hours at the concentrations of 20 and 10 mg/ml can kill both gram positive and gram negative bacteria; What is more, HH 8 hours has the highest activity when compared with other sample. From β-galactosidase assay it was found that when treated with HH 2, 4, 6 and 8 hours at the concentrations of 20 mg/ml the expression of *ftnA* was reduced whereas expression of bfd was increased. Therefore HH might be having potential antimicrobial activity by disrupting iron regulatory pathways. Further studies on bioactivities of HH are needed.

S19/P15

The diffusivity of the environment impacts on the fitness of quorum sensing signal cheats

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Cheating is a naturally occurring phenomenon in bacterial social interactions. This is particularly important because social interactions regulate key virulence factors in a number of pathogenic bacterial species. Many experiments have been conducted to better understand cheat strategies and dynamics in the coordination of bacterial group behaviour by quorum sensing (QS). Yet the focus of these experiments has been on mutants which are incapable of responding to QS signal (signal blind) rather than mutants which do not produce signal. Much is still unknown about the interactions between signal-producing and non-producing bacteria. We do not know whether QS signals act as a public good such that signal-negative mutants could act as social cheats, nor do we know how this interaction is influenced by environmental factors, such as the diffusivity of the signal and the composition of the surrounding media. In this work we analyse how wild-type cells of the opportunistic pathogen Pseudomonas aeruginosa interact with non-signalling mutants (lasI-) while varying the diffusivity of the QS signal through the growth media. We show that in media which requires an intracellular metabolic mechanism only activated by the presence of signal, lasI mutants act as signal cheats and have a higher relative fitness than the signal-producing PAO1 wild-type in mixed cultures. Lowering the diffusivity of the growth media results in the signal molecules being less accessible to the mutants, which in turn leads to lower cheater fitness.

S19/P16

Antimicrobial and antioxidant effects of phenolic compounds extracts of date syrup

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Plant derived products provide a vast source of chemical diversity and have been used as therapeutic agents for the treatment of disease and illness for many years. Phenolic compounds are regarded as nutraceuticals showing varied structural mixtures such as hydrolysable tannins, chlorogenic acids and flavonoids. Phenolic compounds from date syrup were extracted with Amberlite XAD-2 and fractions isolated using RP-HPLC and evaluated for their antimicrobial and antioxidant activities. The antimicrobial activity was screened using Gram positive *S.aureus* and Gram negative *E. coli*. The antioxidant effect was studied using the *in vitro* DPPH free radical scavenging assay and ferric reducing power. The results obtained from the

extraction and isolation of date syrup phenolic compounds detected 3-*O*-caffeoylshikimic acid, caffeic acid, hydrocaffeic acid and *P*-coumaric acid. The antioxidant potential was dependent on date syrup polyphenol extract concentration. In biological assays, results demonstrated that phenolic compounds are inhibitory against *S. aureus* and *E. coli* with an MIC of 30 mg/mL. Isolated phenolic fractions demonstrates significant antibacterial activity in particular hydrocaffeic acid and 3-*O*-caffeoylshikimic acid with mean zones of inhibition at 23.19 mm (± 0.15) and 12.93 mm (± 0.15) for *E. coli* and 21.09 mm (± 0.32) and 13.43 mm (±0.35) for *S. aureus* respectively. However, the antimicrobial activity was more prominent in the whole phenolic extract rather than isolated fractions suggesting the combination of phenolic compounds act synergistically as antimicrobials.

S19/P17

The Role of IQGAP1 in Actin-Based Motility of Burkholderia pseudomallei

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Burkholderia pseudomallei is a Gram-negative bacterium that causes melioidosis, a serious disease of animals and humans in tropical countries, southeast Asia and northern Australia. This pathogen can invade many cell types. It lyses and escapes from the endocytic vacuole after cellular uptake. In the cytosol, it exploits the host cell actin cytoskeleton by a process known as actin-based motility to promote its movement within and between cells. BimA (*Burkholderia* intracellular motility A) is required for this process and is located at the pole of the bacterial cell where actin polymerisation takes place. The mechanism by which BimA subverts the cellular actin machinery is ill-defined. We have used proteomics to identify cellular factors associated with bacteria expressing BimA. IQGAP1 (IQ motif containing GTPase activating protein 1), a scaffold protein that interacts with many different cellular proteins, was detected. Confocal microscopy confirmed that it is recruited to the actin tails of *B. pseudomallei* in infected cells. IQGAP1 plays a role in regulating the actin cytoskeleton and is targeted by several other pathogens. For example, it promotes *Salmonella* invasion into epithelial cells and supports cell attachment and pedestal formation in Enteropathogenic *Escherichia coli*. In this study, we tested whether BimA and IQGAP1 are interacting partners by yeast two-hybrid assay. We found that IQGAP1 does not interact with BimA directly. Using siRNA knockdown we found that the bacterium can still form actin tails in IQGAP1-knockdown cells, however the tails are statistically longer tail with a lower actin density.

S19/P18

Evaluation of Resazurin microtitre plate assay for early phenotypic characterisation of ESBL, AmpC and MBL β-lactamases-producing Enterobacteriaceae

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A rapid dissemination of pathogenic bacteria resistant to multiple antibiotics has become one of the most significant global problems, affecting both patients and health care providers. The effective antibiotics to treat these recalcitrant infections have been decreasing daily resulting in difficulty in selecting appropriate antibiotic treatments. Development of rapid, simple and inexpensive methodology to identify the various resistance mechanisms present in clinical isolates is necessary to inform chemotherapeutic decisions. In the present study a 96-well plate-based resazurin assay, incorporating cefotaxime (CTX) or ceftazidime (CAZ) with specific α-lactamase inhibitors was developed. A total of 42 clinical Enterobacteriaceae producing ESBL, AmpC, MBL α-lactamases, and co-α-lactamase producers were subjected to the resazurin microtiter plate (RMP) assay. The MIC ratios were calculated and interpreted with6 h of incubation. The assay positively detected all 11 ESBLs, 16 AmpCs, 10 MBL, 4 AmpC-ESBL co-producers and 1 ESBL-MBL co-producer. CAZ-based showed better performance in detection of AmpC singly compared to CTX-based, while no difference in characterising ESBL alone. This assay is a promising approach that could be utilised in any laboratories. It could also inform effective treatment and minimise and control the spread of resistant bacterial infections.

S19/P19

Selective conditions for a multidrug resistance plasmid depend on the sociality of antibiotic resistance

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Multiple antibiotic resistance genes (ARG) are frequently clustered on conjugative plasmids and often confer qualitatively different mechanisms of resistance. These multidrug resistance (MDR) plasmids are an important source of clinical resistance. It is critical therefore to understand the selective conditions promoting the spread of these MDR plasmids. Here, we tested how the antibiotic conditions required to select for a multidrug resistant plasmid, RK2, in *Escherichia coli* depended on the mechanism of resistance, specifically whether drug resistance was selfish or cooperative. We observed highly contrasting selective conditions depending upon the sociality of resistance: A selfish drug resistance, efflux of tetracycline, was selected for at

~1% of the minimum inhibitory concentration (MIC), whereas, a cooperative drug resistance, beta-lactamase hydrolysis of ampicillin, was only favoured at antibiotic concentrations exceeding the MIC. When used in combination, dual antibiotic selection was additive and thus selected for the MDR plasmid at concentrations even lower than those observed to be selective under single antibiotic treatments. These results suggest that selfish drug resistances, such as efflux pumps, are likely to play an important role in the dynamics of MDR plasmids in the environment where they can be selected for by very low, sub-MIC concentrations of antibiotic.

S19/P20

The viability of *Burkholderia cenocepacia* isolates is decreased by manuka honey

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Cystic Fibrosis (CF) is a common genetic disorder, the hallmark of which is increased mucous viscosity, allowing colonisation of the lungs by opportunistic pathogens. Multiple antimicrobial treatments and biofilm formation means that pathogens become highly resistant to conventional treatment. Repeated exacerbations lead to irreversible lung damage and decreased lung function, which ultimately can only be resolved by transplant. The presence of some species, such as *Burkholderia cenocepacia*, is associated with poor post-transplant prognosis, limiting long-term treatment options and making its eradication a priority. *B. cenocepacia* is inherently antimicrobial resistant so novel treatment strategies are required.

The antimicrobial activity of manuka honey was demonstrated previously. It also exhibits synergy with several antibiotics, increasing their efficacy. Here, we combined manuka honey with a range of antibiotics commonly used to treat *B. cenocepacia.* The minimum inhibitory concentration (MIC) and fractional inhibitory concentrations of honey and antibiotics were calculated using a panel of isolates. The majority of isolates showed resistance to the antibiotics, but for all the strains tested the manuka honey MIC was <10% w/v. Many isolates also displayed synergy when sub-MIC concentrations of honey and antibiotic were combined.

This work highlights that isolates of *B. cenocepacia* are susceptible to manuka honey. Further to this we have demonstrated its ability to improve the activity of clinically relevant antibiotics. With further *in vivo* investigation there is potential for novel formulations to be developed. Eradication of *B. cenocepacia* from patients has important clinical ramifications and could allow previously ineligible patients to receive a lung transplant.

S19/P22

Characterization of the host-specificity of *Staphylococcus pseudintermedius* surface protein L (SpsL) and its role in the pathogenesis of skin disease

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Staphylococcus pseudintermedius is a major canine skin pathogen and a zoonotic pathogen of humans. The critical host-pathogen interactions mediating *S. pseudintermedius* colonization and infection are not well understood. SpsL is a cell wall-associated protein of *S. pseudintermedius* with affinity for both fibrinogen and fibronectin. Notably, SpsL has a higher affinity for canine versus human fibrinogen but the mechanism underlying this interaction is unknown. In order to examine the fibrinogen binding activity of SpsL, and its role in pathogenesis, we constructed gene deletion mutants of *S. pseudintermedius* lacking fibrinogen-binding activity. Adherence assays comparing the *spsL* gene deletion mutant with the isogenic wild type demonstrated a host-specific binding interaction of SpsL similar to that observed with *Lactococcus lactis* expressing SpsL. ELISA and surface plasmon resonance analyses of recombinant truncated derivatives of SpsL indicate that the predicted ligand-binding N2N3 subdomains of the A-domain are not sufficient for high-affinity host-specific interactions. Ongoing experiments aim to investigate the distinct binding activities of native SpsL presented on the bacterial cell surface, and recombinant SpsL derivatives. The fibrinogen-binding deficient deletion mutant has been investigated in a murine abscess model of infection revealing a role for SpsL in the pathology of skin abscesses. Overall these studies are providing new insights into the role of cell wall-associated proteins in staphylococcal pathogenesis and host-specificity.

S19/P23

Optimization of miRNA extraction from insect infected with *M. anisopliae*

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Entomopathogenic fungi, such as *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Met.) Sorokin, whose natural habitat is soil, have been found to infect a wide range of insect species (Toledo *et al.* 2006). It is important to understand the genetic mechanisms which define host range and, therefore aggressiveness of the fungal pathogens. There is a need to reduce the dependence on chemical pesticides and develop an

understanding which can lead to pathogens targeted to specific pests. The discovery of miRNAs in the *M. anisopliae* suggests early origins as a gene regulator (Zhao *et al.* 2007, Zhou *et al.* 2012). MicroRNA could play a significant role in development or growth through modulation of translation and mRNA stability of the target genes (Bartel, 2009). This work aims to identify, quantify, and determine whether microRNA play a role in the regulation of insect infection by the entomopathogenic- fungus *M. anisopliae*. In addition, to determine what genes are targeted by miRNA regulation to better understand their function in fungal development during infection. The experiments to date have focused on developing the methodology for extracting miRNA and total RNA from the *Metarhizium* infection process. In conclusion, we have compared three commercial miRNA isolation kits for the best performance in extracting miRNA from insects infected with *M. anispoliae*.

S19/P24

Investigation of the role of the *Campylobacter jejuni* Type VI secretion system in secretion of virulence factors and interactions with host cells

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Campylobacter jejuni and *Campylobacter coli* are leading causes of foodborne bacterial gastroenteritis throughout the world. The recent identification of a Type VI secretion system (T6SS) in some *C. jejuni* and *C. coli* isolates has led to the hypothesis that strains harbouring the T6SS have an advantage in terms of pathogenicity or adaptation to the environment. *C. jejuni* strains isolated from South-East Asia have been shown to harbour the T6SS at a relatively high level (up to 70%) (Harrison *et al.*, 2014). To compare the prevalence of the T6SS within European *C. jejuni* strains, whole-genome sequencing analysis of 63 Spanish *C. jejuni* isolates (poultry and urban effluent) identified 14% of strains harbouring all 13 T6SS ORFs (Ugarte-Ruiz *et al.*, 2015). Further analysis of the prevalence of the T6SS identified 56.1% of *C. coli* and 28.8% of *C. jejuni* isolates from retail chickens from Northern Ireland possessing the T6SS (Corcionivoschi *et al.*, 2015). This is the highest observed prevalence of the T6SS to date in Europe. To study the C. jejuni T6SS further, comparison of *C. jejuni* 414 (T6SS +ve) against C. jejuni 11168H (T6SS -ve) showed that the T6SS +ve strain exhibited increased biofilm formation under microaerobic conditions, increased haemolytic activity and increased oxidative stress resistance. Mutation of *tssB* and *tssC* that encode components of the *C. jejuni* T6SS contractile sheath will allow further investigation of the role of the *C. jejuni* T6SS, specifically in relation to survival in the presence of other bacteria and a role in virulence.

S19/P25

The role of the MarR transcriptional regulators RrpA and RrpB in the response of *Campylobacter jejuni* to oxidative and aerobic stress

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Campylobacter jejuni is the leading cause of bacterial gastroenteritis worldwide. *C. jejuni* is microaerophilic with multiple defense mechanisms to tolerate environmental oxygen concentrations and other hostile oxidative stresses. Reannotation of the *C. jejuni* NCTC11168 genome sequence identified two MarR-type transcriptional regulators Cj1546 and Cj1556, which we have designated RrpA and RrpB (regulator of response to peroxide) respectively. Electrophorectic mobility shift assays identified that both recombinant RrpA and RrpB demonstrate auto-regulation, binding to the promoter regions of *rrpA* and *rrpB* respectively. Oxidative stress assays were performed to investigate bacterial sensitivity to hydrogen peroxide (H2O2), cumene hydroperoxide and menadione. Both 11168H *rrpA* and *rrpB* mutants exhibited increased sensitivity to H2O2 stress, whilst the 11168H *rrpAB* double mutant exhibited reduced sensitivity. No differences in sensitivity to cumene hydroperoxide or menadione were observed. Both the *rrpA* and *rrpB* mutants exhibit reduced catalase activity compared to the wild-type strain. RT-PCR indicates that rrpA and rrpB mutants express lower levels of *katA* compared to the wild-type strain. Both mutants also displayed reduced virulence in the *Galleria mellonella* infection model, reduced ability to survive aerobic stress and increased biofilm formation. The *rrpAB* double mutant exhibits wild-type levels of biofilm formation and wild-type levels of virulence in the *G. mellonella* infection model. Differences in *katA* expression between wild-type strain and mutants is been investigated further using qPCR and RNA seq will be used to compare global transcriptome changes. This work aims to identify the mechanisms by which RrpA and RrpB regulate *C. jejuni* oxidative and aerobic stress responses.

S19/P26

GyrA mutation results in fixed alterations to supercoiling and does not appear to impact stress responses

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Antibiotic resistance is a major problem that causes an enormous cost to health and the global economy. Despite some novel molecules targeting Gram positive bacteria, there is a dearth of new antibiotics active against Gram negatives. To prolong use of current drugs we need to understand mechanisms of resistance to inform prescribing practices and drug discovery.

Resistance to quinolones is primarily conferred by mutations in the target loci; DNA gyrase and topoisomerase IV. We have shown that resistance to quinolones also confers a low level of protection to a range of non-quinolone drugs. We hypothesise that altered supercoiling levels, resulting from DNA gyrase mutations, alter expression of stress response genes conferring a generic protective effect.

We analysed the effects of DNA gyrase mutations in *Salmonella* upon supercoiling. Both GyrA Ser83Phe and GyrA Asp87Gly substitutions resulted in patterns of supercoiling which were different to those of the wild-type, and were fixed under multiple conditions.

To determine whether changes to GyrA alter expression of stress responses we fused the promoter regions of genes controlled by different sigma factors to plasmids containing a promoterless *gfp* and introduced them into our *Salmonella* strains. The relative fluorescence of these strains was calculated under a range of conditions by measuring both average fluorescence from cultures and their optical density, and also by using flow cytometry. The data identified differential expression of various stress reporters in the GyrA D87G strain. The role of mutation within *gyrA* in the fitness of Enterobacteriaceae under stress conditions requires further investigation.

S19/P27

Staphylococcus epidermidis clones isolated from preterm neonates presenting with sepsis

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Staphylococcus epidermidis is a Gram-positive bacterium naturally found on human skin and an important opportunistic pathogen linked with neonatal blood sepsis.

Preterm neonates are a highly susceptible patient group for bacterial infections, due to their naive immune status and the invasive procedures to which they are often subjected to in neonatal ICU settings. Rapid detection of blood sepsis and characterisation of the causative pathogen are critical first steps to enable appropriate treatment and improved prognostic outcomes.

As part of the ClouDx-i project consortium, we aim to extend our knowledge of currently circulating pathogenic strains linked with neonatal blood sepsis to inform the continued development of new and improved molecular diagnostic assays. We report results and implications of the establishment of ultra-fast 60-minute procedures, including genome assembly, plasmid detection and genome comparison of several bacterial strains, isolated from preterm neonates at the Royal Infirmary, Edinburgh.

S19/P28

Investigating the bovine caruncular epithelial cell line as a model for *Listeria monocytogenes* invasion of reproductive tissues in ruminants

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Listeria monocytogenes is a food-borne pathogen of major veterinary importance. There has been a 3% increase in bovine abortions caused by this bacterium over the last 17 years. With each abortion costing the dairy farmer around £630, *Listeria*

infections have major welfare and economic consequences. *L. monocytogenes* has a particular tropism for the gravid uterus and while the route of infection of the ruminant placentome is relatively unknown, invasion is thought to be mediated by the interaction of bacterial InlA and InlB with host E-cadherin and c-Met tyrosine kinase receptors, respectively.

This work aimed to compare the ability of abortion, conjunctivitis, meningitis and environmental *L. monocytogenes* isolates to infect the feto-placental barrier. Bovine caruncular epithelial cells (BCECs) were used to model the bovine reproductive tract. The intracellular viability of 14 *L. monocytogenes* isolates was assessed at 2-24 hours post-infection of BCECs. Two abortion isolates and two non-abortion isolates showed significant attenuation at 24 hours post-infection, compared to a well characterized *L. monocytogenes* strain. Three of those isolates were also tested in the gut cell (Caco2) infection model with only two of them also attenuated in those cells, suggesting cell-specific factors are required to infect these cell lines. Three of those isolates also show significantly reduced growth in heart infusion medium. In summary, isolates from cases of abortion appear no better at infecting BCEC cells, and hence reproductive tissues, than isolates from other sources.

S19/P29

Prevalence of Carbapenemase resistant phenotypes and genotypes from a public hospital in Egypt

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Objectives: Carbapenem resistant Enterobacteriaceae (CRE) are resistant to most available antibiotics so they are difficult to be treated, and can be deadly. However, little or no information exists on CRE and related risk factors in developing countries. This research aims to provide an insight into the incidence and spread of CRE in an Egyptian hospital.

Methods: The study included 705 admitted patients to Tanta Teaching hospital, Egypt from April 2014 to April 2015. Samples were cultured on chromogenic agar and recovered isolates were identified at the species level by MALDI-TOF. Antibiotic susceptibility profiles were determined according to the EUCAST guidelines. Detection of genes encoding carbapenemases, such as bla_{NDM}, bla_{VIM}, bla_{KPC}, bla_{OXA-48} was via PCR. Genotyping, plasmid profiling, mechanisms of antibiotic resistance and genome sequencing of the isolates is ongoing.

Results: In total, 873 bacterial isolates were recovered, from which 604 (69%) were Enterobacteriaceae. Carbapenem resistant Enterobacteriaceae (CRE) were 168 (28%) bacterial isolates. The major species among CRE was *Klebsiella pneumoniae* (50%; n=83), followed by *Providencia spp.* (23%; n=39) and *E. coli* (14%; n=24). Central line associated BSI was recorded in (35%; n=59) of the total CRE isolates. CRE genotypes were: bla_{OXA-48}-positive (n=39), bla_{NDM}-positive (n=145), bla_{VIM}-positive (n=28). All of the tested CRE were KPC-negative and 40 isolates processed more than one CRE genotype.

Conclusions: *Enterobacteriaceae* infections are widely distributed among patients in Tanta Hospital and suggests CRE may represent a major clinical challenge for Egyptian hospitals and policy makers.

S19/P30

Investigation of the role of porin proteins in *Salmonella* acquisition of host iron

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Iron is essential for the growth and long-term persistence of most human pathogens, and growth within host tissues is dependent on the bacteria being able to steal iron from the normally secure iron sources, such as the iron-binding proteins transferrin (Tf) and lactoferrin (Lf) (Freestone et al, 2008). It has previously been shown that Lf can bind to the outer membrane porins of some Gram negative bacteria (Sallmann et al., 1999). It is now necessary to study whether other pathogens within the *Enterobacteriaceae* family similarly utilise their porins to bind Lf and Tf and if they do, whether this is a new strategy to acquire iron from their host. The hypothesis of this project is that the OmpD and OmpC porins of *Salmonella* are moonlighting as Tf and Lf binding proteins, and that this novel binding activity is important to the capacity of *Salmonella* to cause infection within its host. The objectives are to mutate both the *ompD* and *ompC* genes in *Salmonella* and analyse their effects on host-iron acquisition and virulence.

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S19/P31

The host metabolite D-serine contributes to niche specificity of enterohaemorrhagic *Escherichia coli*

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The ability of bacteria to sense and respond to their environment is critically important for niche specification. The host intestinal tract provides a challenging environment for pathogenic foreigners that must overcome many barriers before colonisation of a specific site. Enterohaemorrhagic *Escherichia coli* (EHEC) uses a type III secretion system to intimately attach to the colonic epithelium but its expression is tightly regulated in response to signals presented in this environment. We have demonstrated that this process can also be influenced by extraintestinal signals and that this may limit EHEC colonisation in unfavorable sites. One such signal, D-serine, is found in abundance in human urine, is toxic to EHEC and is capable of repressing the type III secretion system at the transcriptional level. Counter intuitively, EHEC also responds to D-serine by increasing its uptake from the environment via a novel transporter YhaO. This transporter is highly conserved in all *E. coli* but regulated uniquely in different pathogenic backgrounds suggesting an EHEC specific adaptation to extracellular D-serine. Additionally we have identified a novel transcriptional regulator (YhaJ) that controls the expression of both *yhaO* and type III secretion in EHEC. This further highlights the adaptive capability of *E. coli* core genes in regulating pathotype specific virulence factors. These studies exemplify the importance of integrating host signals into the transcriptional network of the cell in order to respond appropriately to the current environment.

S19/P32

Construction and Characterisation of CDR20291 ΔPaLoc Mutant Strains for the Study of Binary Toxin Regulation in *Clostridium difficile*.

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Clostridium difficile is a Gram-positive, spore forming, anaerobic bacterium and is the leading cause of hospital-associated diarrhoea in the developed world. The two large monoglycosylating toxins A and B have long been recognised as the major virulence factors of *C. difficile* infection (CDI). The genes encoding these toxins reside on a conserved locus referred to as the PaLoc. So-called hyprevirulent or epidemic strains of *C. difficile* produce a third toxin in addition to A and B, the binary toxin, or CDT, from a different chromosomal locus. Initially the relevance of binary toxin to CDI was unknown but recent advances suggest that CDT plays a major role in *C. difficile* virulence.

The Kuehne group seeks to further investigate the relevance of CDT to *C. difficile* virulence, and to unravel the molecular mechanisms regulating the production of binary toxin. In order to accomplish this effectively, it is necessary to be able to quantify binary toxin production without interference from the main virulence factors, Toxin A and B. This is best achieved by the construction of PaLoc-minus mutants of a hypervirulent strain such as CDR20291.

In this study we have deleted the entire 18.42Kb PaLoc by two-step allelic exchange in CDR20291 Δ*pyrE* and CDR20291 Δ*pyrE*Δ*cdtR* backgrounds. Supernatants collected from cultured mutants contained no detectable toxin A/B as determined by ELISA and did not induce cell-rounding in the Vero cell culture model without prior CDT activation.

To our knowledge, this is the first published description of a clean in-frame ΔPaLoc mutant of *C. difficile*.

S19/P33

The role of *Campylobacter jejuni* fibronectin-binding proteins CadF and FlpA during bacterial interactions with host cells: comparison between 11168H and 81-176 strains

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Campylobacter jejuni is predicted to cause over 400 million human gastrointestinal infections each year. However the mechanisms of infection are still not well understood. Two highly conserved fibronectin-binding proteins CadF and FlpA play a role in the adhesion of *C. jejuni* to intestinal epithelial cells (IECs). CadF and FlpA interact with the extracellular component fibronectin on IECs, mediating activation of a variety of molecular signalling pathways that result in bacterial internalisation. We hypothesise there is strain to strain variation in the expression of CadF and FlpA between the 11168H and 81-176 wild-type strains based on differences in cadF and flpA promoter activity. In both strains, *cadF* is expressed at a higher level than *flpA*. However in 11168H, *cadF* is expressed at a higher level than in 81-176, whereas *flpA* is expressed at a lower level. In fibronectin binding assays, both 1168H and 81-176 *flpA* mutants exhibited reduced binding compared to the respective wild-type strains with the 81-176 *flpA* mutant showing the most significant reduction. Both *flpA* mutants exhibited a reduction in both interactions with and invasion of T84 IECs. However the 11168H *flpA* mutant exhibits an increased level of invasion after 24 hours compared to the 81-176 *flpA* mutant. Infection of *G. mellonella* larvae with live 11168H and 81-176 *flpA* mutants also resulted in reduced cytotoxicity to the larvae compared to the wild-type strains. Further investigations are in progress to study the roles of both CadF and FlpA in *C. jejuni* interactions with IECs.

S19/P34

Investigating the role of the bile salt sodium taurocholate in influencing *Campylobacter jejuni* outer membrane vesicle production, content and function

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Campylobacter jejuni, the leading cause of bacterial acute gastroenteritis in the world, secretes an arsenal of virulence-associated proteins via outer membrane vesicles (OMVs). We have recently shown for the first time (Elmi *et al*., 2015) that *C. jejuni* OMVs interact with and cleave the intestinal epithelial cell (IEC) tight and adherens junction proteins occludin and E-cadherin, promoting enhanced *C. jejuni* adhesion to and invasion of IECs. *C. jejuni* OMVs also impact on IEC antimicrobial immunity, inducing innate immune and antimicrobial defence responses, and are cytotoxic to IECs and *Galleria mellonella* larvae. *Vibrio cholerae* senses the bile salt sodium taurocholate (ST) as a host signal to co-ordinate the activation of virulence associated genes. We hypothesised that ST may also act as a host signal to influence the biological functions of *C. jejuni* OMVs. Physiological concentrations of ST do not have an inhibitory effect on *C. jejuni* growth until the early stationary phase. Co-culture of *C. jejuni* with 0.1% or 0.2% (w/v) ST enhances OMV production, increasing both lipid and protein concentrations. *C. jejuni* ST-OMVs possess enhanced proteolytic activity and exhibit a different protein profile compared to OMVs isolated in the absence of ST. Further studies are in progress to investigate whether the enhanced proteolytic activity of ST-OMVs is associated with increased cytotoxicity and immunogenicity as well as further disruption of the IEC barrier function. Our preliminary data offer new insights and supports the hypothesis that ST influences *C. jejuni* OMV-associated virulence proteins, via the modulation of OMVs production, content and function.

S19/P36

Epidemiology and Clinical Outcomes of Gram negative Multi-drug Resistant Organisms causing Neonatal Sepsis in Pakistan

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Sepsis is one of the leading causes of neonatal mortality and morbidity. Carbapenems are the primary drugs for treatment of sepsis caused by multi-drug resistant strains but the carriage of carbapenemases by resistant organisms lead to treatment failure. A total of 192 Gram negative strains isolated neonates admitted in Neonatal unit, PIMS Hospital, Islamabad, were included in the study where the prevalence of carbapenem resistant organisms causing neonatal sepsis and their imapct on patient outcome was analyzed. Antimicrobial susceptibility was done by disk diffusion and E- test methods. PCR screening of isolates was performed for the detection of beta lactamases genes. The clinical detailed data of length of hospital stay, underlying disease, therapy along with the outcome was also studied to access the clinical burden of these isolates. Majority of the tested isolates were multi-drug resistant with highest resistance against cefotaxime (70%). Of 192 gram negative strains, 77 were positive for MBL as tested by E-test. Of these 77 MBL positive isolates, 72 were positive for blaNDM-1. KPC-2 gene was detected in 2 isolates of Klebsiella pneumoniae. One isolate had co-existence of blaNDM-1 and blaIMP. Mortality rate was 72.3%. Of these 69% died in early neonatal period and 31% died during late neonatal period. Out of expired neonates, 62% were cases of pre-term birth. The presence of carbapenemases in gram-negative bacteria make these infections difficult to treat, especially in neonates. A proper protocol for antibiotic stewardship and guidelines for treating and preventing these infections should be introduced in the hospitals.

S19/P37

Characterisation of clinical bovine *Listeria monocytogenes* isolates - virulence gene expression and cell wall integrity.

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Listeria monocytogenes, a rod shaped Gram positive facultative anaerobic bacterium, infects a range of hosts including humans and cattle. Infection can lead to abortion, meningitis, septicaemia and death. Clinical disease caused by *L. monocytogenes* is a significant public health concern, and leads to economic losses in dairy and beef industries. Internalins mediate the invasion of *Listeria* into a host cell via E-cadherin and Met, and are encoded by virulence associated genes *inlA* and *inlB*.

Isolates where characterised from healthy (n=1) and clinically affected bovines, diseased samples included conjunctiva (n=3), brain (n=1), aborted material (n=6) as well as environmental isolates (n=2).

Relative gene expression of internalins was determined using Quantitative (q)PCR and normalised to the reference gene *tufA*. All isolates expressed both internalins but *inlA* expression was significantly lower in an environmental isolate ($p<0.05$), potentially contributing to the isolates reduced ability to infect human gut cells (Caco-2) and bovine caruncular epithelial cells (BCEC).

To investigate if cell wall and cell membrane integrity affects the virulence of *L monocytogenes*, isolates were challenged with cell wall acting antibiotics and cell membrane acting antimicrobial peptides (AMP). An isolate from a healthy eye, determined to be attenuated when infecting conjunctiva and BCEC showed significant higher sensitivity to cell wall actin antibiotics. Other isolates showed transient growth arrest or bacterial lysis in response to AMP, suggesting reduced membrane integrity, however there was no correlation with the ability to infect and replicate in various different infection models.

S19/P38

Proteomic profiles of plasmid mediated antibiotic resistance in *Escherichia coli*

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This study investigated how different antibiotics treatments effect the protein abundances produced from a multi-drug resistant plasmid (pEK499) and *Escherichia coli* host chromosome. The proteomic profiles of a multi-drug resistance plasmid (pEK499) and chromosome of *Escherichia coli* under antibiotic stress were investigated using the Q-Exactive mass spectrometer. The bacteria were exposed to antibiotics, for which a resistance gene was present on the plasmid (ampicillin and cefotaxime) at high concentrations or sub-MIC concentrations for those, which the bacteria were susceptible (imipenem and ciprofloxacin). The plasmid contains the *aac(6') Ib-cr* quinolone helper resistance gene. The plasmid proteomes and chromosome proteomes of these bacteria under stress were compared to the same *E. coli* with no antibiotic stress.

The results indicated that the presence of antibiotic resistance proteins from the plasmid was not dependent on the presence of the corresponding antibiotic. The quantities of blaTEM and blaCTX-M-15 proteins were similar under antibiotic stress or no antibiotic stress. The relative abundances of chromosomal proteins varied in comparison to the control from increases in 27 proteins (ampicillin) to increases in 98 proteins (ciprofloxacin) and decreases in protein abundances of 28 proteins (imipenem) to 80 proteins (ciprofloxacin). There were no common proteins to all antibiotic exposed or control bacteria. Proteins involved in gene expression were the most frequently identified metabolic pathway or process. In conclusion the variations between the relative abundances of chromosomal proteins in different samples, appears to be antibiotic specific but not antibiotic class specific and thus could indicate specific antibiotic-induction pathways or metabolic processes.

S19/P39

The ovine interdigital microbial community with a focus on footrot

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Ovine footrot is characterised by the under running and separation of the hoof from the foot and degeneration of the horn. Footrot is a major welfare concern for sheep farmers worldwide. Untreated cases impact negatively on the health and productivity of affected animals resulting in loss of income. Footrot occurs after physical damage to the interdigital skin allowing for the colonisation by *Dichelobacter nodosus*, which leads to separation of the hoof horn from the underlining tissue. A second bacterium, *Fusobacterium necrophorum*, is thought to exacerbate the severity and prolong the persistence of footrot. There are, however, multiple other bacteria present at each stage of the disease, which could also exacerbate the infection or alter conditions favourably for pathogenic species.

In order to understand the differences between the microbial communities present in healthy and footrot affected feet, a 16S rRNA amplicon survey was conducted using interdigital skin biopsies. The resulting data was used in a k-mer based taxonomic assignment pipeline to allocate the sequences to their specific bacterial genera.

Both healthy and footrot affected samples showed shared commensal and environmental bacteria, such as *Staphylococcus*, *Bacillus*, *Enterococcus* and *Salmonella*. However, when comparing the footrot associated microbial community to the clinically

healthy samples there were two noticeable additions of *Mycoplasma* and *Treponema*, which are also found in contagious ovine digital dermatitis and bovine interdigital dermatitis. *Fusobacteria* was also found in a greater abundance whilst *Dichelobacter* was present in both clinically healthy and footrot affected samples at similar levels.

S19/P40

Multi locus sequence typing of digital dermatitis treponemes reveals frequent between host transmission events.

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Treponema species are known to cause periodontal disease in humans, and digital dermatitis (DD), which is characterised by severe lesions on the feet of cattle, sheep, goats and wild elk. Substantial global animal welfare issues and economic losses to farmers are caused by DD. Cultivable DD treponemes are classified into three phylogroups, based on their 16S rRNA gene sequence; Treponema medium, Treponema phagedenis and Treponema pedis. The 16S rRNA genes sequence has previously proved unsuccessful for differentiating isolates from different hosts.

Isolation of DD treponemes is complicated by contaminating bacteria from foot lesions, and their highly fastidious nature. In this study, we undertook multi locus sequence typing (MLST) of 118 DD treponeme isolates from different species, from nine different countries. Seven different housekeeping genes were amplified by PCR, and sequenced, revealing a low level of diversity among strains within each phylogroup.

Isolates within sequence types (ST) were generally not confined to specific host species, suggesting that the same bacterial STs are able to infect different animals. Some ST's were confined to a farm or local area, suggesting between host transmissions, whereas others had a more global distribution.

Of the three phylogroups, T. pedis was the most variable, with lower levels of diversity seen in the other two phylogroups. Compared to other spirochaetes, treponemes are not comprehensively differentiated by MLST, suggesting full genome analysis may be more useful for taxonomic purposes. The relative similarity between strains from different host species suggests continued vigilance and surveillance of DD treponeme infections is required.

S19/P41

Structural basis for the Myf and pH6 fimbriae mediated tropism of pathogenic Yersinia

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Three pathogenic *Yersinia* species assemble host cell adhesion fimbriae via the chaperone/usher pathway: *Y. enterocolitica* elaborate Myf fimbriae from MyfA subunits, *Y. pseudotuberculosis* and *pestis* instead form the pH6 antigen from PsaA subunits. To elucidate the role of these fimbriae in tissue tropism of pathogenic *Yersinia*, we performed high-resolution structural studies of MyfA and PsaA complexed with binding determinants of their receptors. We found that, as PsaA, MyfA specifically binds to galactose, a terminal residue of ganglioside receptors. Additionally, PsaA exhibits three binding sites for choline using a unique tyrosine-rich surface absent in MyfA. Thereby, Myf fimbriae of *Y. enterocolitica* may facilitate intestine colonization by recognizing ganglioside receptors on enterocytes, whereas pH6 antigen of *Y. pseudotuberculosis* and *Y. pestis* may also promote colonization of lung by binding to phosphatidylcholine lipids abundant in alveolar cells.

S19/P42

Novel inhibitors of bacterial topoisomerases with potent activity against multidrug resistant Gram-positive and fastidious Gram-negative bacteria

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The prevalence of antibiotic resistance has created a need for novel antibacterials to treat infections caused by bacterial pathogens such as drug-resistant methicillin-resistant Staphylococcus aureus (MRSA) and Neisseria gonorrhoeae. To address this unmet medical need, Redx Pharma has discovered a novel series of small-molecule inhibitors of the bacterial type II topoisomerases. This family of enzymes, which includes DNA gyrase and topoisomerase IV, facilitates negative supercoiling and stress relief of bacterial DNA during replication and transcription. Representative compounds from this series, REDX04139, REDX05931 and REDX06345 showed antibacterial

activity against a range of Gram-positive and fastidious Gram-negative organisms such as S. aureus (MIC = 0.015, 0.06, and 0.5 µg/mL, respectively) and N. gonorrhoeae (MIC= 0.12, 0.12, and 0.5 µg/mL, respectively). Compounds retained potency against quinolone-resistant MRSA and multidrug resistant N. gonorrhoeae. Acceptable cytotoxicity profiles (>64 µg/mL) were determined in an in vitro assay using the HepG2 cell line. REDX05931 demonstrated a balanced, and superior inhibition of supercoiling and decatenation activity of S. aureus DNA gyrase and topoisomerase IV (1.31 ± 0.33 µM, and 0.21 ± 0.01 µM, respectively) compared with ciprofloxacin (16.96 ± 10.81 µM, and 4.35 ± 0.57 µM, respectively), and AZD0914 (6.37 ± 5.56 µM and 4.55 ± 0.86 µM, respectively). Frequencies of resistance (<10-10) are consistent with a balanced dual-targeting inhibition. Similar to ciprofloxacin, REDX05931 was bactericidal, reducing viable cells by 4.4 log units over 24 h at 16×MIC. This series of small-molecule bacterial topoisomerase inhibitors have promising biological properties, warranting further investigation and development.

S19/P43

Displacement of pathogens by an engineered probiotic is a multi-factorial process that depends on attachment competition and interspecific antagonism

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Pathogen attachment to host cells is a key process during infection, and inhibition of pathogen adhesion is a promising approach to the prevention of infectious disease. We have previously shown that Multivalent Adhesion Molecules (MAMs) are abundant in bacterial pathogens and commensals, mediate early attachment to host cells and are important virulence factors. Here, we investigate the efficacy of an engineered probiotic expressing a commensal MAM on its surface in preventing pathogen attachment and pathogen mediated cytotoxicity in a tissue culture infection model. We analyze the individual contributions of adhesion and interspecific antagonism on overall outcome of infection for a range of different pathogens, by comparison with a fully synthetic adhesion inhibitor. We find that the potential of the probiotic to outcompete the pathogen is not always solely dependent on its ability to hinder host attachment, but depending on the pathogenic species, may also include elements of interspecific antagonism, such as competition for nutrients and loss of fitness due to inter-species aggression.

S19/P44

Evaluation of flagellar gene expression in *Salmonella enterica* serovars identifies species wide differences in flagellar gene regulation.

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Salmonella enterica is an enteric bacterial species that colonises plants, animals, reptiles and humans. Whole genome sequence analysis of *S. enterica* generates a phylogenetic tree comprising of three clades: A1, A2 and B. These 3 clades encompass the known 2,500 serovars used to type *Salmonella* during clinical outbreaks of salmonellosis. *S. enterica* exploits the bacterial flagellum to be motile in liquid environments and over surfaces. The enteric flagellar system of *S. enterica* is organized into a transcriptional hierarchy of three promoter classes: class I - driving expression of the master regulator flhDC; class II - needed for hook and basal body subunit production; and Class III - dependent of the flagellar specific sigma factor s28 essential for expression of the filament subunit flagellin. Our understanding of flagellar gene regulation in *S. enterica* stems from seminal work conducted over the last 30-40 years in the serovar Typhimurium. In this study, we asked how does activation of flagellar gene expression across the species *S. enterica* compare to serovar Typhimurium?

We will show a comparison of motility phenotypes, flagellar gene expression and bioinformatics analysis of flagellar genes in 23 serovars representative of the 3 clades of *S. enterica*. Our data suggests that the timing of flagellar gene expression for all serovars is similar, but the magnitude of flagellar gene expression varies significantly. Further analysis of specific serovars will be presented that suggests the changes in the magnitude of flagellar gene expression across serovars is dependent the regulation of FlhD4C2 activity.

S19/P45

Optimisation of a Three-dimensional Airway Epithelial Model of the Ovine Respiratory Tract Grown at an Air-Liquid Interface

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The culture and differentiation of respiratory epithelial cells at the air-liquid interface (ALI) allows for the development of a cell stratum, which is highly representative of the tissue *in vivo*. Lung tissue from domesticated animals such as cattle and sheep can be readily obtained from abattoir material and thus represents a cost-effective and ethical alternative to infection studies using live animals. The use of tissues from mammalian hosts which have respiratory epithelia of similar physiological structure to human epithelia also makes such models appropriate for the study of pathogens which infect the human respiratory tract. We have carried out an extensive study examining a variety of critical factors which regulate the efficiency of airway epithelial cell differentiation during ALI culture. Growth substrates, medium constituents and gas composition of the culture environment were manipulated and the degree of differentiation assessed by histological analyses, immunofluorescence microscopy and scanning electron microscopy. By comparing a wide variety of the factors that affect differentiation, we have selected the optimum growth conditions that allow for the development of a well-differentiated epithelial layer, which comprises the key respiratory epithelium cell types including ciliated epithelial cells, mucus-producing goblet cells and basal stem cells. This model will prove particularly useful in the study of relevant bacterial and viral pathogens of the ovine respiratory tract.

S19/P46

Long term evolution of Pseudomonas aeruginosa populations in biofilms

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Pseudomonas aeruginosa is an opportunistic pathogen that chronically infects cystic fibrosis (CF) lungs and chronic wounds by forming biofilms. Emergence of phenotypically diverse isolates within *P. aeruginosa* biofilms has been already reported. However the dynamics between various clones evolved within the biofilms and the possible effects of these clones on the progression of chronic infections and treatment outcomes are poorly understood. Here we tested how the *P. aeruginosa* strain PAO1 evolves in biofilms over 50 days using a bead biofilm model and a synthetic sputum media. Our long term evolution experiment showed that within biofilms, the emergence of distinct *P. aeruginosa* morphotypes evolve when compared to planktonic control experiments. We observed higher levels of biofilm formation and loss of social traits in P. aeruginosa populations evolved in biofilm treatments compared to the PAO1 ancestor. Furthermore, we found that the biofilm evolved population and various selected morphotypes are resistant to certain antibiotics despite no antibiotics being added to the selection experiment. Studying the possible interactions between selected morphotypes, we found both competition and cooperation amongst the selected morphotypes. Investigating interactions between evolved morphotypes will help inform on new strategies for treatment and control of biofilms and also provide explanations as to how and why phenotypic diversity and antibiotic resistance evolves.

S19/P47

Species wide analysis in *Escherichia coli* to determine the impact of IS element insertion in to the *yecG-flhDC* intragenic region on motility

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The flagellar system of *E. coli* is organized into a transcriptional hierarchy strictly dependent on the expression of the flagellar master regulator *flhDC*. It is well established that *E. coli* tightly regulates flagellar gene expression via flhDC transcription, translation and protein levels. Uropathogenic *E. coli* isolates are highly pleiotropic with respect to their motility phenotype. Previous studies of domesticated laboratory strains suggest that *flhDC* transcription is dependent upon the presence or absence of an IS element at various sites within the *yecG-flhDC* intragenic region. Screening UPEC clinical isolates found only 1 to contain a yecG-flhDC IS insertion. Consistently up to 10 % of *E. coli* genomes assessed exhibit a *yecG-flhDC IS* insertion. This suggested IS insertion is not a dominant factor in dictating motility in *E. coli* isolates.

Bioinformatic analysis of the *yecG-flhDC* region showed that the region reflected the phylogenetic order of *E. coli.* However, 3 dominant sequence types of the *yecG-flhDC* region mapped to clades A/B1, B2 and D/E. We have asked what impact replacing the *yecG-flhDC* intragenic region with model sequence types has upon motility? We will present an analysis of *flhDC* promoter activity and promoter switching experiments in various *E. coli* strains. Our data suggests that even though the *yecG-flhDC* region is prone to IS element attack, other factors dictate the regulation of *flhDC* transcription across *E. coli* as a species.

S19/P48

Surviving and Thriving in the airway: How the Arginine Specific Autotransporter Aminopeptidase could promote *P. aeruginosa's* success as a Cystic Fibrotic pathogen

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Pseudomonas aeruginosa is highly problematic for Cystic Fibrosis (CF) patients. More than 80% of patients are infected before adulthood. Once colonisation occurs, it is difficult to eradicate due to it being resistant to the host's immune defences and many of the antibiotics currently available for treatment. This pathogen's superior resilience has been attributed to its formation of a biofilm inside the airway mucus layer. Due to the high density of bacterial cells within a biofilm, oxygen and energy sources are in high demand. Studies show that oxygen is quickly depleted from airway mucus colonised with *P. aeruginosa*. Alternative energy pathways using available resources therefore need to be employed. Several metabolic pathways exist for arginine metabolism in *P. aeruginosa*. These can generate energy in aerobic and anaerobic conditions. A source of arginine could therefore support maintenance of a stable biofilm. A protein called the Arginine Specific Autotransporter Aminopeptidase (AaaA) releases arginine from the end of peptide chains, which the bacterium can then utilize. The *P. aeruginosa* Δ*aaaA* mutant is less successful at chronically infecting burn wounds than its wild-type counterpart. This study builds on existing knowledge of AaaA. We have investigated whether it is present in CF isolates, under what conditions it is expressed and how it is regulated. Inhibition of AaaA *in vivo* could significantly attenuate the virulence of *P. aeruginosa*, facilitating successful treatment of infection in CF.

S19/P49

The effect of the short chain fatty acid derivative sodium propionate on adherent invasive *E. coli*

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The use of antibacterial compounds to prolong the shelf-life of food products has increased dramatically in recent years. While the addition of these compounds has been of benefit economically, a related increase in inflammatory bowel disease (IBD) has been reported. Antibacterial compounds including short chain fatty acids (SCFA), such as propionic acid (PA) are used to treat a variety of food stuffs including meat, bread and mayonnaise. PA is commonly added to bread on an industrial scale and chickens are reared on drinking water and feed supplemented with PA. In the present work, our data indicates that the AIEC E. coli strain LF82, commonly found in patients suffering from IBD, is able to utilise PA to out-compete commensal E. coli strains. This may confer an advantage to AIEC strains under conditions not favourable for other bacteria. Our data also indicates that when grown anaerobically, PA-adapted LF82 form biofilms to much greater levels than both the WT and commensal E. coli. This could indicate increased virulence through an ability to persist in an environment which is unfavourable for other strains. PA-adapted LF82 display higher adherence and invasion efficiency than the WT strain. Comparison of the genome sequence of WT LF82 to that of PA-adapted LF82 identified potential adaptive mutations in 14 genes. These genes encode proteins with functions including acid stress response, adhesion, motility and structural integrity. In conclusion, these results indicate that PA is utilised by the AIEC strain LF82 and may play an important role in its pathogenicity.

S19/P50

An unexpected chest cause of chest pain

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A 56 year old previously fit and well Caucasian male presented with a 5 day history of severe diarrhoea and vomiting associated with mild central chest pain. On arrival he was hypotensive, tachycardic and febrile with a mildly tender abdomen. His ECG showed saddle shaped ST elevation and bloods demonstrated an elevated troponin of 130 ng/L (normal<14), profound hypophosphataemia at 0.27 mmol/L (0.87-1.45) with CRP 39 mg/L (0-5) and normal full blood count and differential. An X-ray of the abdomen revealed thickened small bowel loops. He developed atrial fibrillation with fast capture, which resolved with correction of fluid balance and electrolyte abnormalities. A bedside echocardiogram was unremarkable.

Stool cultures were positive on PCR and culture for *Campylobacter coli*. One bottle out of three sets of blood cultures taken was positive for *Moraxella osloensis*. The patient responded to treatment with Azithromycin and recovered well. Although increased exposure to *M. osloensis* through home composting and organic farming was speculated, in the absence of more common gut organisms in blood culture to suggest translocation, this was felt to be a contaminant. A diagnosis of perimyocarditis and gastroenteritis secondary to *C. Coli* was established and treatment with Azithromycin was effective. Repeat transthoracic echo was unremarkable.

Perimyocarditis secondary to *Campylobacter* is extremely rare, and mainly seen with jejuni isolates. However the overall incidence of campylobacter infection is rising and it is therefore important to be alert to this possible complication.

S19/P51

Prevalence and virulence characteristics of Enteroaggregative *Escherichia coli* in a case- control study among patients from Iran

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Enteroaggregative *Escherichia coli* (EAEC) is an important agent of diarrheal diseases worldwide. The role of EAEC virulence factors in the clinical outcome of infection is not completely defined. This case–control study investigated the prevalence of EAEC, its virulence genes and antimicrobial resistance profile of adult patients with and without diarrhea attending three different hospitals in Zanjan, Iran. A total of 550 individual stool specimens (350 from diarrheal patients and 200 from patients without diarrhea) were collected. One hundred forty one EAEC isolates were identified by HEp-2 cell assay and PCR. EAEC isolates were detected with slightly higher frequency in patients with (27.7%) than in patients without (22%) diarrhea ($P > 0.05$). The EAEC genes *aggR*, *aap* and *pet* were identified more frequently in case patients compared with controls ($P < 0.05$). The most EAEC isolates in the diarrheal patients had two or more virulence genes compared with those without diarrhea ($P < 0.05$). EAEC isolates exhibited high level resistance to amoxicillin (82.3%), co-amoxiclav (78%), aztreonam (73.8%), tetracycline (66.6%) and ceftazidime (63.8%). Also, 53.2% of isolates were resistant to at least three different classes of antimicrobial agents and considered as multidrug resistance. These results indicate a high prevalence and heterogeneity of gene profiles of EAEC in diarrheal and control patients and suggest that the presence of *aggR*, *aap*, and *pet*, the number of genes present and antimicrobial resistance may be markers for more virulent EAEC isolates.

S19/P52

Amino Acid Utilisation by *Clostridium difficile*

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The carbon and energy metabolism of the human pathogen *Clostridium difficile* is poorly understood. Deeper insights may be gained by metabolic analyses using liquid chromatography-mass spectrometry (LC-MS) platforms, but such experiments are best performed in well-defined growth media. The aim of this study was therefore to develop a fully defined growth medium and to compare amino acid utilisation for eight *Clostridium difficile* strains (CD630Δerm, DH196, R20291, EK15, EK28, R12801. L26, 017 Serotype F). The data generated suggest that cysteine, glutamine, isoleucine, leucine, serine, threonine and tyrosine are preferentially utilised, both in the presence and absence of glucose. Several other amino acids, including asparagine, glycine, phenylalanine, proline and valine were also utilised but to a lesser extent. Thus, LC-MS profiling confirmed that this organism derives most of its carbon and energy from the fermentation of glucose and a selected range of amino acids. Of particularly interest was the observation that all strains except 017 Serotype F appeared to have initially utilized alanine and shortly afterward accumulate it again during the exponential phase of growth. The reason for this was not established but may be explained by the use of pyruvate as an amino acceptor during the degradation of preferentially fermented amino acids. Our results form the basis for further investigation of the previously reported link between amino acids metabolism and toxin production in *C. difficile*.

S19/P53

Utility of Propidium monoazide-qPCR for evaluating the non-viable component of bacterial signals in clinical samples

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One of the drawbacks of pathogen- or 16S-directed qPCR analyses of clinical samples is their inability to differentiate between signals from viable and non-viable bacteria. Propidium monoazide (PMA) is a high affinity photoreactive DNA-binding dye that causes selective suppression of DNA amplification from dye-permeant cells and from free DNA. After photoactivation PMA is covalently bound to DNA.

In our studies on chronic obstructive pulmonary disease (COPD) we have been concerned to differentiate signals from “dead” and “live” bacteria in sequential samples to better understand the community dynamics in the sputum microbiome. Here we report our results evaluating the influence of growth phase and storage on PMA-treated to untreated qPCR signals from respiratory pathogens and COPD samples. We found species, growth phase and storage related effects with *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *P. aeruginosa* and *S. aureus* and discuss the impact of our results on the interpretation of signals from COPD samples.

S19/P54

In vitro determination of the activity of the antimicrobial peptides colistin, bicarinalin and BP100 against *Acinetobacter baumannii*

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Acinetobacter baumannii is an opportunistic pathogen, part of the ESKAPE group, implicated in nosocomial infections including pneumonia and septicaemia. *A. baumannii* infections are difficult to treat due to its ability to form biofilms and resistance to multiple antimicrobials. This study compared the activity of two antimicrobial peptides (AMPs), bicarinalin and BP100, against *A. baumannii* with colistin, a polymyxin antibiotic currently used as a treatment of last resort for multi-drug resistant (MDR) *A. baumannii* infections.

Minimum bactericidal concentration (MBC) results were ~0.5µg/ml for colistin and 4µg/ml for both bicarinalin and BP100 against *A. baumannii*. Biofilm dispersal results demonstrated bicarinalin and BP100 had significantly greater biofilm removal potential than colistin. At peptide concentrations of 1024µg/ml, biofilm eradication was ~50% for colistin but 65% and 83% for bicarinalin and BP100 respectively.

Atomic force microscopy (AFM) illustrated dramatic changes to cell size and conformation at concentrations above the MBC. Scanning electron microscope (SEM) images visualised the reduction in biofilm coverage and altered cell conformation as the peptide concentration increased. Confocal laser scanning microscopy (CLSM) images and bio-volume quantification using the nucleic acid stain SYTO9 further reinforced the evidence of peptide activity and indicated that the AMPs bicarinalin and BP100 were significantly more effective than colistin against *A. baumannii* biofilms.

The results demonstrated the potential for these AMPs as therapeutic alternatives to currently used treatments for A. baumannii infections. If bicarinalin and BP100 prove to cause less side effects than colistin in patients they could be developed for the treatment of MDR A. baumannii infections.

S19/P55

Novel Genes of Methicillin-resistant *Staphylococcus aureus* Involved in Beta-lactam Resistance

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Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to represent a serious issue in clinical environments around the world. b-lactam resistance in MRSA depends on the transpeptidase activity of low-affinity penicillin binding protein, PBP2A, which is encoded by *mecA* gene. In addition, resistance also requires certain 'auxiliary factors' such as *fmtA*, *tagO* et al. Using transposon mutagenesis, we recently have identified two novel auxiliary factors and named *auxA* and *auxB* respectively. Transposon insertion into both genes causes a 2-32 fold reduction in the minimum inhibitory concentration (MIC) of Cefoxitin, Oxacililin, Ceftazidime, Cephradine and Meropenem, and complementation restores MICs back to the wild type form. Additionally, transduction of transposon mutations into different backgrounds of MRSA including livestock-associated MRSA (LA-MRSA), community-associated MRSA (CA-MRSA) and Hospital-acquired MRSA (HA-MRSA) leads to beta-lactam MIC reduction. These suggest both *auxA* and *auxB* genes are required for b-lactam resistance in various MRSAs. The identification and and characterization of novel auxiliary factor genes deepen our understanding on the resistance mechanism and may provide new strategy to treat MRSA infection.

S19/P56

Evidence for cross-talk in DNA mobilisation related to two integrative and conjugative elements in *Klebsiella pneumoniae*

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The genome of *Klebsiella pneumoniae* HS11286 carries 9 genomic islands, including two integrative and conjugative elements (ICEs), ICE*Kpn*HS-1 and ICE*Kpn*HS-2; the former belongs to the ICE*Kp1* family and the latter is currently unassigned. Type IV secretion systems (T4SS) are one of the major modules of these ICEs and are responsible for conjugative transfer of nucleoprotein complexes. Preliminary evidence led us to study the interaction between the two distinct T4SSs, from two different ICEs, in DNA mobilisation. A plasmid containing *oriT* of ICE1 (pACYC184-*oriT1*) was constructed to facilitate conjugation assays. Initial work revealed that deletion of ICE2 led to a three-fold reduction in trans-conjugants of the ICE1 marker plasmid. Subsequently we demonstrated that deletion of the *mob* ortholog in ICE2 produced a similar defect which could be complemented in trans. Further selective deletions were used to improve the dynamic range of the test system and to identify the genes contributing significantly to the crosstalk. We also report data on transfer of the native chromosomal ICEs and discuss the potential mechanisms underpinning our results.

S19/P57

Displacement of Stable Bacterial Plasmids by a Self-Transmissible pCURE Plasmid as a Means of Reducing Antibiotic Resistance Gene Load

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Antibiotic resistance is becoming one of the greatest scientific and medical challenges of the 21st century. However our arsenal to combat this surge is limited to a small number of different classes of antimicrobial drugs. The importance of plasmids carrying antibiotic-resistance genes as targets for potential therapies has not been fully exploited and technologies that specifically address the plasmid burden could yield useful therapies. We have shown that by harnessing the innate biology of plasmid replication and maintenance functions we can stably repress replication and eliminate plasmids from a population. This concept has been taken further and using the broad-host range IncP1 RK2 backbone, a conjugative curing plasmid, pCURE100, was constructed. Curing experiments on solid and liquid media were carried out and showed successful elimination of the test plasmid from a chosen population in the absence of any antibiotic selection. On the basis of these experimental results, we are now developing a safe to use and easily administered conjugative, broad-host range plasmids that can efficiently target antibiotic resistance carrying plasmids within the gastrointestinal tract of humans and animals, and improve the treatment options for infections caused by multi-drug resistant Enterobacteriaceae

S19/P58

Investigation effect of antimicrobial peptide in combination with antibiotics against pathogenic intracellular bacteria associated with Cystic Fibrosis

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Compromised mucociliary clearance in the lower respiratory tracts of the respiratory system of Cystic Fibrosis (CF) patients makes them vulnerable to opportunistic infections such as *Staphylococcus aureus* (MSSA) and *Pseudomonas aeruginosa.* Moreover, lungs can be inhabited by the free-living amoebae, *Acanthamoeba* which feeds on bacteria. Potentially it harbours engulfed microorganisms protecting them from extrinsic factors. Problems related to the infections are compounded by the absence of effective chemotherapeutic agents. Magainin II is naturally arising and synthetic membrane-active peptide, a chemical that have been revealed to be disruptive against range of Gram-positive and negative bacteria as well as *Acanthamoeba castellanii*. Silver cations (Ag+) display a lethal impact on many micro-organisms comprising bacteria, viruses and algae, at the same time being less cytotoxic for human cells.

Our previous studies have revealed that various concentrations (200 μM; 100 μM; and 50 μM) of Magainin II exhibited an antimicrobial effect on *Acanthamoeba*, A549 cell line, extracellular and intracellular bacteria. In this study, the aim was to investigate the susceptibility of the above microorganisms using sub-lethal doses of Magainin II alone, in combination with antimicrobial agents such as silver nitrate, and two antibiotics vancomycin and ciprofloxacin. The antimicrobial effect of Magainin II on the survival of *Acanthamoeba* was dose dependent with sub-lethal dose of 6.5 μM. However, adding both vancomycin and ciprofloxacin with Magainin II (6.5 μM) had no effect. Both MSSA and *Pseudomonas* required higher sub-lethal dose (12.5 μM) when inside *Acanthamoeba*. The result was changed dramatically in the presence of antibiotics.

S19/P59

Mechanosensing of fluid shear induces virulence in Escherichia coli O157:H7

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Enterohemorrhagic Escherichia coli O157:H7 is a food-borne pathogen transmitted via the fecal-oral route and can cause bloody diarrhea and hemolytic uremic syndrome (HUS) in the human host. Although a range of colonization factors, Shiga toxins and a type III secretion system (T3SS) all contribute to disease development, the locus of enterocyte effacement (LEE) encoded T3SS is responsible for the formation of lesions in the intestinal tract. While a variety of chemical cues in the host environment are known to up-regulate LEE expression, we demonstrate that changes in physical forces at the site of attachment are required for localized, full induction of the system and thus spatial regulation of virulence in the intestinal tract. We show that different force vectors (attachment, fluid shear) can be integrated to result in a dose-dependent output of LEE induction. This constitutes a novel mechanism of virulence regulation in EHEC, but here we will further discuss our results in the context of other studies, which frame mechanosensing as a new paradigm for virulence regulation in bacterial pathogens.

S19/P60

The expired microbiome sampled by use of face masks

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We have been evaluating the use of modified face masks as a means of sampling the microbial content of exhaled air particles. We are using this approach to study respiratory diseases such as tuberculosis, pneumonia and chronic obstructive pulmonary disease. We have shown that the approach is effective in detecting tubercle bacilli in TB patients (PLoS One 9:e104921) and are now assessing its value in providing samples from the lower airways in other conditions.

We report here our preliminary results from a study of over 100 samples from healthy volunteers and consenting patients with respiratory disease. Subjects were invited to wear adapted face masks for periods of up to 1 hour while undertaking specified respiratory efforts including coughing, normal breathing, instructed tidal breathing and reading out loud. As with our TB study, samples were acquired on gelatine filters from which we then obtained extracts for DNA and protein analysis. Bacterial 16SrDNA-directed analyses indicate contamination rates of between 104 and 106 copies per hour of phyla including Firmicutes, Gamma- and Beta-proteobacteria, Actinobacteria and Bacteroidetes. Evidence of mask contamination with particles from the lower and upper respiratory tracts has been obtained from these phylogenetic assignments and from quantifying surfactant protein A and albumin. Mask sampling provides important new opportunities to characterise the microbial content of human expired air.

S19/P61

Potential use of *Lactobacillus fermentum* 3872 as an anti-campylobacter agent

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Due to the rise of multidrug resistant form of pathogenic microorganisms, alternative intervention tools are in urgent need. One option is to employ beneficial (probiotic) bacteria, which could compete with pathogens for host cell attachment cites and elicit antibacterial activity. *Lactobacillus fermentum* strain 3872, which revealed outstanding probiotic activity, is a good candidate for such studies. Genome sequencing of this strain revealed a novel plasmid (pLF3872) containing a gene, encoding a collagen-binding proteins (CBP). The plasmid was not found in any other strains of the species. The chromosomal genome sequence of this strain (2.3 Mb) was found to contain other genes potentially contributing to its beneficial effects, such as those encoding a mucus binding protein and other adhesins, as well as a bacteriocin-encoding gene not found in other sequenced genomes of these bacteria. ELISA-based attachment experiments revealed competition of *L. fermentum* 3872 with *Campylobacter jejuni* strain 11168H for binding to collagen I, which is a ubiquitous structure making up the gastrointestinal tract. The results suggest that *L. fermentum* 3872 can potentially be used for competitive exclusion of *Campylobacter jejuni*, which is the most important gastrointestinal pathogen.

S19/P62

Infection and disease mechanisms of TRH-producing *Vibrio parahaemolyticus*

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Globally, *Vibrio parahaemolyticus* is the leading bacterial cause of illness associated with the consumption of shellfish. Changing climatic conditions has led an increasing number of outbreaks in Europe and other temperate regions. However, some of these infections are caused not by the thermostable direct haemolysin (TDH)-containing "pandemic" clone that emerged from India, but by a growing number of isolates that contain the TDH-related haemolysin, TRH.

We are using a range of *in vitro* and *in vivo* assays to describe the virulence mechanisms of clinically-relevant TRH+ *V. parahaemolyticus* isolates. We used polarised epithelial monolayers on Transwell filters to study the impact of the strains on epithelial barrier function. All strains induced a significant reduction in transepithelial resistance, a measure of epithelial barrier integrity, suggesting that the barrier was no longer intact. We also found TRH+ *V. parahaemolyticus* isolates causing earlier and more severe disease than TDH+ isolates in an infection model. Like TDH+ isolates, TRH+ strains preferentially colonised the distal small intestine and caused profound tissue destruction. We are currently using a range of histologic and immunofluorescence techniques to describe the kinetics, distribution and host cell response to TRH+ isolates in order to better understand how these pathogens cause disease. Finally, studies using the *Galleria mellonella* larvae model support the idea that TRH-containing isolates differ in their virulence to TDH-containing isolates. Overall, our studies indicate that factors other than TDH, contribute to species pathogenicity and as such, better 'markers' to detect pathogenic *V. parahaemolyticus* may be needed to protect public health.

S19/P63

A cross sectional survey: Attitude towards adult vaccination in Karachi-Pakistan

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As per the estimates of National Interview Health Survey(NHIS)-2012, adult vaccination is extensively ignored and a very less number of adults could receive recommended vaccine doses. Thus, a cross sectional study was conducted to evaluate the knowledge and attitude towards adult vaccination programs among the adult citizens of Karachi,Pakistan.The survey covered about 54.28% of the total areas of Karachi,Pakistan.A questionnaire comprising of 11 questions was designed and a total of 500 individuals ranging from 11-61 years of age participated in the study. Responses to each question were statistically analyzed and the associations between different variables were established using SPSS 16. According to the results, majority (93.4%) of the individuals considered vaccines safe for health and more than 80% agreed that adult immunization is as necessary as child immunization however,a significant difference ($p<0.05$) was noticed between the vaccination schedule follow up rates during childhood and adulthood and only a few individuals could receive the recommended adult vaccines.A significant association ($p<0.05$) was also found between the frequency of infections and the factors that may affect the efficiency of immune system such as sleeping disorders,smoking,persistent depression/ anxiety,alcohol intake and the use of anti-depressive drugs/ steroids/cortisones.Results showed that a number of reasons may increase the reluctance of people towards any vaccination program such as the cost,risk of quality assurance and requirement of booster doses.Thus,the study emphasizes on the need of awareness programs for adult vaccination as the lack of awareness in this sector may render masses susceptible to serious & life threatening infections.

S19/P65

Outer Membrane Proteins profiling identified unique OMPs potentially linked with acid tolerance of Neonatal Meningitic Pathovar *Cronobacter sakazakii* CC4

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Background: *Cronobacter* is a member of the Enterobacteriaceae family. The *C. sakazakii* CC4 clonal lineage is associated with neonatal meningitis and has been isolated from both powdered infant formula (PIF) processing plants and from PIF. Outer membrane proteins (OMPs) are important for *C. sakazakii* survival in environmental conditions and in human hosts. This study analysed the OMP profiles of *C. sakazakii* isolates to investigate whether OMP profiles differed in CC4 vs. non-CC4 strains.

Materials and Methods: Infant formula was adjusted to pH 3.5 with HCl, and the growth curves of 13 *C. sakazakii* CC4 and non-CC4 strains were determined in the formula at 37°C. Bacteria were also cultured in BHI broth at pH 6 and 3.5, and the OMPs were extracted and separated by SDS-PAGE. The protein band patterns were analysed using GeneSnap™ and BioNumerics™ software.

Results: CC4 strains were more acid-resistant than non-CC4 strains to culture at pH 3.5, which is the pH of the neonatal stomach. CC4 strains also had more OMP bands than non-CC4 strains at pH 3.5, including bands in the following MW ranges: 99–93, 60–65, 50–53, 35–30 and 15–19 kDa. These data suggest that CC4 strains may have OMPs that are involved in bacterial survival and in adapting to changing environmental conditions. The OMP of interest will be further characterised in the future.

S19/P66

Effects of Manuka Honey on Cystic Fibrosis Associated Pathogens.

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In modern medicine, antimicrobial resistance is increasing and our antibiotic lineup is wavering. It is crucial that we identify novel therapeutics if we are to avoid pre-antibiotic era death rates. Manuka honey has a proven track record of antimicrobial prowess when treating surface wound infections, however its potential in other areas of infection is limited. Our current remit is to ascertain the feasibility of manuka honey as an effective therapeutic agent for the treatment of microbial infections associated with cystic fibrosis (CF). Using standardized antimicrobial susceptibility testing methods, the efficacy of manuka honey against a wide range of CF-pathogens (such as *Burkholderia cepacia* complex *spp.*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, and *Staphylococcus aureus*) was investigated. Manuka honey was shown to inhibit all of the clinical and reference isolates tested ($n = 76$) with concentrations <20% (w/v). Additionally, sub-inhibitory manuka honey concentrations in combination with conventional antibiotics were tested against a range of *P. aeruginosa* reference panel isolates ($n = 28$). For many of the isolates, combination treatment lead to additive inhibitory efficacy, with some observable synergistic interactions against some isolates. Here we have shown the potential of manuka honey against a wide range of CF-pathogens, even when diluted to sub-inhibitory concentrations (in the presence of conventional antibiotics), suggesting manuka honey is an ideal novel therapeutic, or that it could be used to reinvigorate the wavering line up of effective antibiotics. The overall potential for manuka honey to be formulated specifically for the treatment of CF-pathogens is highly promising.

S19/P67

Superoxide Dismutase Activity is Important for Oxidative Survival, Biofilm Formation and Mucoviscosity of *Klebsiella pneumoniae*

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Klebsiella pneumoniae is the causative agent of several nosocomial and community acquired infections. Some of its virulence determinants have been identified but it is not known how it copes with damaging effects of reactive oxygen species. Superoxide dismutase (SOD) is responsible for removal of toxic superoxide radicals, and *K. pneumoniae* genome contains three superoxide dismutase genes coding for Mn-, Fe- and CuZn- co-factored SODs, *sodA*, *sodB*, and *sodC*, respectively. This work is designed to evaluate inducibility of sod genes during oxidative stress, and the importance of each SOD in oxidative survival, biofilm formation and mucoviscosity of *K. pneumoniae*.

Lambda Red system and Flp-recombinase mediated excision were used to construct markerless isogenic *sod* mutants. Single, double, and triple SOD mutants were characterized phenotypically through growth studies in oxygenated environment, by biofilm formation assay using polystyrene microtitre plates, and by microscopy. Finally, mucoviscosity of mutants and wild type were determined by glucouronic acid quantification.

Phenotypic characterization of single, double and triple isogenic SOD deficient mutants indicated that *sodB* alone or in combination with *sodA*, have a major impact on total SOD activity and both *sodA* and *sodC* are inducible. We also found that Mn-SOD is essential for growth in oxygenated environment, biofilm formation and mucoviscosity, which is linked to *K. pneumoniae* virulence.

S19/P68

Isolation and Molecular Characterization of *Riemerella anatipestifer* Isolated from Outbreaks in Egypt

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Riemerella anatipestifer is considered one of the most important threats for ducks and ducklings worldwide. In the present study, isolation and molecular characterization for *R. anatipestifer* based for full length outer membrane protein (OmpA), sequencing and phylogeny followed by prediction for the ligand binding sites on the surface of the immunogenic protein (OmpA). Blast result for the obtained nucleotide sequences revealed the circulation of different serotypes among the Egyptian duck industry indicating progressive adaptation and evolution of *R. anatipestifer* among ducks and ducklings. Phylogeny was conducted based on full length *Omp*A gene that classifies the diversity of *R. anatipestifer* worldwide into two main lineages (lineage 1 and lineage 2); each lineage diversified into three main clusters and confirmed by 3D prediction. Till now, at least 21 *R. anatipestifer* serotypes have been identified in spite of the molecular techniques regarding its pathogenicity and antigenicity are not well understood. Our study reports the first genotyping of *R. anatipestifer* based on an immunogenic protein (OmpA) and confirm the co-circulation of different *R. anatipestifer* clusters in the Egyptian flocks. Our isolates were belonging to lineage 1 (cluster I and II) and linage 2 (cluster I and III). These findings represent a start to what should be an expanded investigation of the genetic diversity of *R. anatipestifer* at national and regional levels to better understand its evolution dynamics, distribution and the genetic relatedness.

Key words: Ducks, *Riemerella anatipestifer*, OmpA, Phylogeny, Evolution.

S19/P69

Phenotypic and genetic characterization of *Salmonella* isolated from slaughterhouse in Jiangsu, China

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Samples from slaughtered pigs and the slaughtering environment were collected from three slaughterhouses in Yangzhou, Jiangsu province, China from October 2012 to July 2013. The positive identification rates of *Salmonella* in slaughtered pigs and the environmental samples were 46.6% and 48.8%, respectively. S. Derby was most prevalent in slaughterhouses, but other serovars like *S.* Typhimurium, *S.* Meleagridis and *S.* Anatum were also widespread. Antimicrobial susceptibility testing revealed that 32 and 131 different MDR patterns were found among the strains from the environment and slaughtered pig samples, respectively. Fifty-six isolates of *S.* Derby and 16 strains of *S.* Typhimurium were characterized by the technique of pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Xba* I. 35 and 11 PFGE patterns were generated among the selected isolates. Four isolates of *S.* Derby isolates with the same pattern (PF26) were isolated from cooling water, evisceration and carcass, suggesting that cross contamination occurred between the environment and the slaughtered pigs. Six *S.* Typhimurium in cluster 1 with the same ST type (ST19) came from different parts of the slaughtered pig, which could have occurred because of horizontal transmissions along the slaughtering process. The same PFGE patterns of *Salmonella* were found in both samples from carcasses in the slaughterhouse and in the Yangzhou pork market, proving that *Salmonella* had spread from the slaughterhouse to the pork market. In conclusion, our study demonstrate that serious cross contamination occurred in Yangzhou slaughterhouses and can contribute *Salmonella* contamination in pork sold in the local public market.

S19/P70

Characterization of a novel antimicrobial system

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Antimicrobial resistance is increasingly serious threat to global health. Consequently, to address resistance mechanisms and to develop non-antibiotic based therapies capable of bypassing resistance acquisition is of high priority. Peroxidase-like antimicrobial systems offer a promising solution to this. Here, we report a novel biocidal complex which is produced by the reaction between ionic oxidizible salts - iodide and thiocyanate - in the presence of hydrogen peroxide as an oxidation source. Such a reaction provokes the generation of bactericidal reactive oxygen and iodine species. In this study we demonstrated that the iodo-thiocyanate complex is a potent bactericidal agent against Gram- (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram+ (*Staphylococcus aureus* and methicillin resistant *S. aureus*) bacteria, causing rapid death of bacteria in planktonic and biofilm modes. Furthermore, the attempts to introduce resistance in these bacteria towards the "killer cocktail" proved to be not successful. Though the knowledge on the mode of action of the antimicrobial composite is still incomplete, there are indications that its antimicrobial activity is most likely the combinational effect of the powerful species capable of oxidizing the essential biomolecules of bacteria, and perhaps is a result of simultaneous events. The use of this composition may provide an effective and easy method for killing potential pathogens, as well as for disinfecting and removing biofilm contamination.

S19/P71

Enhanced decontamination of *C. difficile* spores on surfaces via the synergistic action of 405nm light and disinfectants

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The ability of *C. difficile* to form spores which can survive for prolonged periods causes significant environmental contamination problems. 405nm light has wide antimicrobial activity against vegetative bacteria, and is being developed for environmental decontamination within hospitals. As expected, spores are more resilient to inactivation. This study aims to establish whether spore susceptibility can be enhanced by combining 405nm light with low concentration chlorinated disinfectants: sodium hypochlorite, Actichlor and Tristel.

Spore suspensions were seeded onto surfaces including PVC, stainless steel and vinyl flooring. Disinfectant was added to the surface, and the samples were then exposed to 405nm light at irradiances of ~0.2-225mWcm-2. Control samples were exposed to 405nm light alone, and disinfectants alone, to establish the sporicidal activity of each agent, and to demonstrate the synergistic effect when combined.

Results demonstrated increased sporicidal activity of 405nm light and low-concentration sodium hypochlorite and Actichlor against *C. difficile* seeded on vinyl flooring and PVC surfaces, with approximately 3-log10 reductions achieved with up to 66% lower doses than achieved with light alone. Tristel demonstrated limited synergy on vinyl and PVC, whilst all three disinfectants demonstrated minimal synergy on stainless steel. Results are also reported for lower intensity light, as used in the clinical environment.

In conclusion, the sporicidal efficacy of 405nm light is enhanced when used alongside chlorinated disinfectants. Further research could potentially lead to the use of lower strength chlorinated disinfectants in combination with 405nm light to provide enhanced decontamination of *C. difficile* spores in the clinical environment.

S19/P72

Can *Staphylococcus* Develop Tolerance to Antimicrobial 405 nm Light?

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There is increasing interest in the use of 405 nm light for decontamination due to its antimicrobial properties and safety advantages over UV-light, however little is known about the potential for bacteria to develop tolerance following exposure. This study investigates whether tolerance is evident in *Staphylococcus aureus* after repeated sub-lethal exposure to 405 nm light, and if repeated exposure affects antibiotic susceptibility. A methicillin-sensitive *S.aureus* (MSSA) type strain (NCTC 4135) and an epidemic methicillin-resistant *S.aureus* (EMRSA-15) underwent 15 cycles of repeated sub-lethal exposure to 405 nm light at an irradiance of 60 mW/cm2. Each sub-lethal exposure delivered a dose of 108 J/cm2, with survivors subsequently enumerated. Inactivation kinetics and antibiotic susceptibility (using the disc diffusion method) were established after 0, 5, 10 and 15 sub-lethal exposures. No evidence of tolerance to 405 nm light was seen in MSSA after 15 sub-lethal exposures; no significant difference in inactivation was achieved after the 1st and 15th sub-lethal exposures, with 92% and 90% reductions in population, respectively. A similar result was found with EMRSA-15, with 90% and 93% inactivation achieved after the 1st and 15th sub-lethal exposures, respectively. No change in antibiotic susceptibility was detected in any of the light-exposed cultures, with the maximum change in the zones of inhibition being ±3 mm. Overall, this study demonstrates that both MSSA and EMRSA-15 did not become tolerant to 405 nm light inactivation following repeated sub-lethal exposures and that repeated exposure is unlikely to affect susceptibility to antibiotics.

S19/P73

The antimicrobial activity of the traditional herbal remedy *Prosopis africana*

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With the steady increase in global antimicrobial resistance rates, concomitant with a recent paucity of novel antibiotic discovery, it is essential that new antimicrobial compounds are discovered to prevent a return to the pre-antibiotic era over coming decades. Consequently, the aim of this work was to assess the antimicrobial properties of one particular candidate, the traditional African herbal remedy Kembo (*Prosopis Africana*). Crude Kembo extracts were prepared by steeping the material in 50% aqueous ethanol, and then subsequently evaporating the ethanol. Cultures of both aerobic - *S. aureus*, methicillin-resistant *S. aureus, E. coli*, *Ps. aeruginosa* and *S. epidermidis* - and anaerobic - *P. acnes* - bacterial species were exposed to the crude extract to perform an MIC and MBC assay. In addition some preliminary quantitative and semi-quantitative biofilm assays were performed using the Kembo extract. It was found that the Kembo extract had both bacteriostatic and bactericidal activity against all of the bacterial species tested, although the degree to which the Kembo was active was variable for each of the bacterial species. Furthermore, Kembo was found to be more active against *P. acnes* than against the aerobic species tested. Finally, in the preliminary biofilm studies the Kembo extract was observed to inhibit biofilm production by MRSA. As the bacterial species tested in this study can be pathogenic, these results hold promise for further investigation into the potential of this herbal remedy in a pharmaceutical context.

S19/P74

Multiple clones and low antimicrobial resistance rates for *Salmonella enterica* serovar Infantis in Greece 2007 to 2010

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Molecular analysis was used to study whether the emergence *S.* Infantis in Greece resulted from different biotypes or a successful spread of one clone. All the *Salmonella enterica* serovar Infantis (*S.* Infantis) strains isolated under official control programs in Greece during a 4year period (2007-2010) were studied. A total of 40 non-epidemiologically related strains were examined, 23 of human origin, 16 from food animals and one from food. Antimicrobial susceptibility testing was performed using agar dilution method in a panel of 20 antimicrobials and PFGE was performed using *XbaI* as restriction endonuclease. High rates of antimicrobial resistance to streptomycin were observed, but only among human isolates (48%) indicating that may have been acquired through horizontal gene transfer and not by selective pressure. All the isolates from food animals and foods were punsusceptible; resistance to cephalosporins was also not detected. Pulsed Field Gel Electrophoresis revealed 31 *XbaI* distinct pulsotypes among the 40 strains with 60% overall similarity reflecting diversity. Three main clusters were constructed, using an 85% cut of value, clusters A, B and C consisting of 14, 6 and 16 isolates respectively. In all clusters strains from poultry and human origin were present indicating transmission through the food chain. Point source of transmission was not detected as multiple reservoirs of the serovar seems to be present in Greece during 2007 to 2010. The results obtained shows that diversity was noticed among *S.* Infantis populations and multiple reservoirs mainly in poultry were present in Greece during the study period

S19/P75

An Assessment of Household Drinking Water as a Reservoir of Antimicrobial *Resistant-Escherichia* coli and *Salmonella* species.

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Source and stored drinking water from selected household in three States of Nigeria were examined for the presence of *E.coli* and *Salmonella* species. *E. coli* and *Salmonella* sp. were characterized using standard methods, subjected to antibiotics susceptibility tests and extended spectrum beta lactamase (ESBL)-resistant genes were detected using polymerase chain reaction. One hundred and eighty water samples were collected from the different households and in total, Twenty-five (25) *E. coli* and (20) *Salmonella* species were isolated and confirmed. The profile of their Antibiotic susceptibility showed that all the *E.coli* (100%) were resistant to Augmentin (aug), ceftazidime (cez), cefixime (cex), cefuroxime (cefu), tetracycline (tet), nalidixic acid (nal), ciprofloxacin (cro), gentamicin (gen), ofloxacin (ofl), nitrofurantoin (nit) and co-trimoxazole (cot). Only 4 (16%) *E. coli* were sensitive to cefotaxime (cefo). All the 20 (100%) *Salmonella* sp. showed resistant to aug, cez, cef, tet and cot whereas 30%,

25% of the *Salmonella* sp. were sensitive to cro and ofl respectively while 5% were sensitive to gen and cefo.. Of all the *E. coli* isolates that were screened for ESBL resistance genes, 3 were positive for the TEM genes while only1 had SHV gene. However, none of the *Salmonella* species were positive for any of these genes. This study concluded that improper storage of drinking water could be a source of household exposure to antibiotic resistant *E. coli* and *Salmonella* sp.

S19/P76

Novel sequence types of *Campylobacter jejuni* isolated from farm associated Norway rats

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Carriage of *Campylobacter* by farm animals, in particular poultry and the importance of this in the transmission of *Campylobacter* to humans is well documented. The route of transmission of *Campylobacter* to turkey and chicken flocks is less clear. The possibility that Norway rats (*Rattus norvegicus*) may act as a reservoir of *Campylobacter* and contribute to contamination of poultry flocks was considered. Thermophilic Campylobacter strains were isolated from rat faecal pellets from eight different farm sites across South-East of England. The majority of isolates were *C. jejuni* but also included *C. coli*, *C. lari*, *C. hyointestinalis*, *C. upsaliensis*. Whole Genome Sequencing (WGS) of 143 isolates and analysis of extracted MLST profiles identified presence of sequence types (STs) commonly associated with farm animals and human disease including ST21, ST42 and ST45. Interestingly there were two additional dominant novel clades of *C. jejuni* frequently isolated over 2 years from all farms. Properties of these unusual *C. jejuni* strains will be presented and the significance of their association with Norway rats considered. This study shows that farm-associated rats are frequently colonised by common STs of *C. jejuni* as well as previously unrecognised types that may have evolved in association with this host.

S19/P77

Characterization of and genomic insights into a biofilm forming and arsenic resistant bacterium *Lampropedia cohaerens* CT6T isolated from the hot springs of Manikaran, India.

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In an attempt to explore the bacterial diversity at stressed niches, we isolated, characterized and sequenced a novel biofilm forming, arsenic tolerant bacterium *Lampropedia cohaerens* CT6T from the microbial mats of a hotspring located at Manikaran, India. Strain CT6T, an aerobic, catalase positive but oxidase negative bacterium showed 95.4% similarity to *Lampropedia hyalina* ATCC 11041T. A polyphasic approach was used to further characterize the isolate. Transmission electron microscopy revealed non-flagellated cells with wavy margins. Polyamine analysis revealed putrescine, spermidine and the beta-proteobacterial specific 2-hydroxyputrescine as the major polyamines. The major polar lipids were phosphatidylethanolamine and phosphatidylglycerol and the major quinone was ubiquinone-8. Based on the genotypic, physiological and chemotaxonomic properties, strain CT6T was considered as a novel species for which the name *Lampropedia cohaerens* sp. nov. was proposed. To further our understanding, we sequenced the genome of this *Comamonadaceae* bacterium using Illumina HiSeq 2000 platform generating about 900 fold coverage. Filtered raw reads were assembled using Abyss v 1.3.5 generating a 3.1 Mb genome assembled into 41 contigs. Genome annotation of this GC rich bacterium (G+C%=63.5) revealed 2,823 coding sequences. An interesting genetic repertoire was harboured by strain CT6T including genes responsible for imparting resistance to arsenic, copper, cobalt, zinc, cadmium and magnesium. Consistent with its niche, genes associated with biofilm formation were harboured. Additionally, pyrroloquinoline-quinone production, isoquinoline degradation and mineral phosphate solubilisation genes present can possibly confer survival benefits at stressed niches. Genome data generation and analysis have provided significant insights into the genome of this yet unexplored genus.

S19/78

Harnessing the power of bacterial bioluminescence to shine a light on antimicrobial resistance.

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Pseudomonas aeruginosa is a common microbial contaminant relating to product recalls in the home and personal care (HPC) industry. Its large genome encodes the ability to survive with minimal nutritional levels, exhibit multiple metabolic capabilities and mediates the innate resistance that may allow it to survive HPC preservative exposure. Due to its dominance as a health care pathogen, little is known about industrially isolated P. aeruginosa strains and their mechanisms of preservative resistance, or the bacterial pathways these agents may inhibit.

The overall research aim is to map genetic and metabolic pathways associated with preservative insusceptibility. Transposon mutagenesis was used to generate a bank of industrial P. aeruginosa mini-Tn5-luxCDABE biosensors for investigating responses to HPC preservatives. Light emission alterations were measured to identify biosensors responding after 24 hour exposure to benzisothiazolinone (BIT), a preservative widely incorporated into household cleaning products. To date 3000 biosensors have been screened with BIT and 81 of these mapped to genes of interest by sequence analysis of DNA flanking the transposon insertion sites. Mutations in pigment production pathways, transcriptional regulators, transporter proteins and a Ferrous-iron efflux pump have been linked to biosensors altering light emission in response to BIT. This research has demonstrated the utility of the reporter transposon bank and identifies BIT as a preservative amenable to screening with industrial P. aeruginosa biosensors.

Future work will develop a biosensor panel to predict bacterial responses to preservatives, enabling industry to develop preservation systems which enhance product sterility, whilst also minimising possible resistance development.

S20/P1

Structural and biophysical studies of genome assortment in Avian Reovirus

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Reoviruses are pathogenic double-stranded RNA viruses that contain 9 - 12 genomic segments encapsulated in a double-layered concentric capsid. In order to successfully replicate and package progeny viruses within viroplasm a complex array of protein-RNA and RNA-RNA interactions are required. In avian reovirus these are facilitated by the non-structural protein σNS. While a possible mechanism has been proposed for genome assortment there is a lack of supporting structural data. Through a combination of structural and biophysical (in particular X-ray crystallography, fluorescence and SAXS) techniques, a preliminary structural model for σNS-RNA complex is being developed.

S20/P2

Whole Genome Analysis of Monovalent human Rotavirus vaccine G1P[8].

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In July 2013, a vaccination campaign was introduced into the UK for children between 2-3 months of age using an oral live attenuated monovalent human rotavirus G1P[8]. Since the introduction of this vaccination campaign, there has been a 70% reduction in the number of reported cases of rotavirus infection. Full genomic sequencing using the Illumina platform was performed on six independent batches of vaccine and four bulk samples to determine homogeneity between vaccine lots. Nineteen single nucleotide polymorphisms (SNP) were found compared to the references (JX943604-JX943614). Of the nineteen SNP identified over the 11 dsRNA segments, thirteen where present in all six batches at similar frequencies, mean variant frequency between batches differed by no more than 7%, ten were present in VP4, one SNP was at a variant frequency greater than 50% and the rest where expressed at a frequency less than 17%. For the remaining six SNP, while these where present in some but not all batches, there variant frequencies where below 3.5%. Our deep sequencing data shows that the six independent batches are comparable to the reference on Genbank with only one virus sequence change at nucleotide position 1091 in VP4 being different from published literature. A number of minor variants have been identified between the reference and the batches suggesting that the vaccine stocks are not completely homogeneous. It is important that vaccine manufacturer's monitor these variants to ensure they do not accumulate during the manufacturing process, as these may have a knock-on effect on vaccine efficacy.

S20/P3

Genetic Stability Assessment of Monovalent Lamb Rotavirus Vaccine G10P[12] by Whole Genome Analysis

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In China approximately 60 million doses of the live-attenuated Lanzhou rotavirus vaccine (derived from rotavirus-infected lambs) have been administered to children younger than 5 years of age since licensure of the product in 2000. To assess the genetic stability of the vaccine, viral RNA was extracted from six batches of vaccine and PCR amplicons produced for all 11 segments. These were deep sequenced and data compared to the seed vaccine reference genome, which had been generated through cloning and Sanger sequencing. There were 53 single nucleotide polymorphisms (SNP) identified in the vaccine, 35 of which were common to all batches with 15 present only in VP4. Seven SNP occurred at a frequency greater than 50%, 3 at 25-50% and 25 at 1-25%. The mean variant frequency for any SNP was no more than 7% between vaccine batches. The remaining 18 SNP were not present in all batches and the frequencies varied between 1-37%.

Our data indicate that there is ≥ 97% nucleotide identity between sequences of the vaccine batches and the seed vaccines, which were used to generate the reference sequences. The latter will be analysed by deep sequencing to determine the extent to which polymorphism has been underestimated in the seed vaccines.

Though the relevance of the changes with regard to immunogenicity and virulence cannot be ascertained in this study, it would appear important to monitor all genetic mutations since an accumulation of SNP could result in the appearance of revertant strains or less effective vaccines.

S20/P4

SUMOylation In Bluetongue Virus Infected *Culicoides* KC Cells.

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The Small Ubiquitin-like Modifier protein, SUMO, covalently links to other cellular proteins (post translation) altering their function and cellular distribution. In mammalian cells / virus systems SUMOylation can modulate both virus replication and host cell innate immune responses.

Bluetongue is one of the economically important 'global' diseases of ruminants. Outbreaks of disease in northern Europe have been caused by Bluetongue virus (BTV) serotype 8 in 2006-2009 and again in 2015, and Schmallenberg virus (SCV), both of which are transmitted by biting midges- *Culicoides spp*. Recently the genome sequence of KC cells (a cell line derived from *Culicoides sonorensis* - the North American vector for BTV) has been made available to us, allowing identification of enzymes involved in the SUMOylation pathway in the midge.

We have demonstrated that SUMOylation can modulate the replication of BTV in mammalian cells and that the SUMOylation pathway is active in mosquito cells. Here, we present novel data identifying the SUMOylation pathway within midge cells. The role of SUMOylation in BTV infection of KC cell is under investigation and is reported here. A better understanding of the SUMOylation pathway in the midge will support further exploration of the *Culicoides* cell's response to BTV, potentially identifying anti-viral targets within the insect vector.

S20/P5

A Method to Assess Cross-Protection Against Multiple Rotavirus Strains Mediated by IgA in Vaccine Recipients

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Rotavirus, the most common cause of severe diarrhoea in infants, is responsible for approximately 500,000 deaths a year, globally. Numerous vaccines have been developed and introduced into national immunisation programs and have been shown to be effective at reducing severity of infection by multiple strains. However, no correlate of this cross-protection has ever been validated. Genetic re-assortment of rotavirus outer capsid genes VP7 and VP4 has resulted in a high diversity of serotypes. Detection of antibody against one rotavirus serotype does not necessarily correlate with heterotypic protection. A likely mediator of cross-protection is IgA raised against the conserved inner capsid protein, VP6. We are developing a new assay utilising a baculovirus produced, EGFP RNA-tagged rotavirus VLP. Levels of anti-VP6 in clinical samples will be tested for their ability to block assembly of the VLP with recombinant outer capsid proteins *in-vitro*. Reduced fluorescence of recipient MA-104 cells would imply presence of anti-rotavirus VP6 and could serve as a useful correlate of protection against multiple rotavirus strains.

S21/P1

Investigation of the variability of autophagy responses during influenza virus infection

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Autophagy is a cellular pathway wherein damaged organelles, infectious organisms and cellular components are degraded. Target molecules in the cytoplasm are engulfed in a double-membraned vesicle, the autophagosome, which then fuses with a lysosome. Influenza A virus (IAV) exploits autophagy and has been shown to both positively and negatively regulate it in order to evade host defence responses and to possibly promote proliferation and replication. The IAV M2 protein induces autophagy initiation whilst also inhibiting autophagy by blocking the fusion of the autophagosome to lysosome, resulting in an accumulation of autophagosomes in the cell. However, previous studies have seen significant variability in IAV-mediated autophagy induction, inhibition and effector functions. Thus, we have combined M2 transfection experiments with IAV infections using multiple different strains and host cells. Stable cell lines in which autophagy can be specifically activated and inhibited have also been used. We then investigated markers of autophagy, including LC3-II and p62 by microscopy and immunoblotting. The LC3-II complex forms during autophagy induction and marks autophagosomes and autolysosomes. M2 effects on autophagy were interpreted through observation of LC3-II redistribution in mammalian cells, whereas p62 is targeted for destruction via the autophagosome and thus enables monitoring of autophagy flux. Our investigations have shown that the induction and M2-inhibition of autophagy are highly variable and significantly affected by a range of parameters, including cell line, virus strain, infection method, time and dose.

S21/P2

Molecular mechanism of membrane scission driven by the influenza A virus M2 protein.

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Influenza A is an enveloped, negative sense RNA virus which causes annual epidemics and major pandemics. Assembly and budding of new viral particles is a complex and multistep process, of which many aspects remain unclear despite many years of research. The Influenza virus M2 protein is a homotetrameric transmembrane protein, containing three domains: ecto domain, transmembrane domain and cytoplasmic tail (CT). In the final stage of budding it has been shown that M2 mediates membrane scission through an amphipathic helix (AH), which is formed by the first 16 amino acids of the protein's CT; however the exact mechanism by which membrane scission is triggered was not known. Here we show that the M2 AH is unstructured in solution, but forms an AH upon lipid binding. The AH preferentially binds to the membranes with high positive membrane curvature, which are detected by sensing lipid packing defects. There are many cationic residues in the polar face of the M2 AH which could interact with anionic lipid headgroups; however, charged interactions do not affect M2 AH interactions with membrane. When inserted into the membrane, the M2 AH increases lipid ordering, induces positive membrane curvature and mediate membrane scission. Together these activities mediate the final step of Influenza virus budding, allowing for release of newly formed virions in to the environment.

S21/P3

HA1-2–fljB vaccine candidate induces immune responses against pandemic swine-origin H1N1 influenza virus in mice

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In 2009 a novel pandemic swine-origin influenza A (H1N1) virus emerged as the first official influenza pandemic of the 21st century, and caused a public emergency of international concern. Vaccination is the primary strategy for the prevention and control of influenza epidemics. The poor immunopotency of many vaccine antigens is a major barrier to the development of an effective vaccine against influenza. Flagellin, a Toll-like receptor 5 (TLR5) ligand, has been used as an adjuvant to enhance the immunopotency of vaccines in preclinical studies. Here, we developed a recombinant candidate vaccine, HA1-2–fljB, in which the globular head of the hemagglutinin (HA) antigen (residues 62–284) of the A/swine/Jangsu/38/2010 (H1N1) virus w*as fused genetically to the N-terminus of* Salmonella typhimurium fljB. The recombinant HA1-2–fljB protein was expressed efficiently in *Escherichia coli*, and the immunogenicity and protective efficacy of recombinant HA1-2–fljB were evaluated in a mouse model. Immunization with HA1-2–fljB elicited robust IgG antibodies and neutralizing antibodies and completely protected the mice against infection by swine-origin influenza A/swine/Jangsu/38/2010 (H1N1). These results suggest that HA antigen placed at the N terminus of flagellin is also an excellent starting point for creating a fusion HA1-2–fljB protein as candidate vaccine, and the recombinant HA1-2–fljB protein will contribute to the development of a more effective vaccine against swine-origin influenza virus infection.

S21/P4

Segment 2 from influenza A(H1N1)pdm09 viruses confers temperature sensitivity on HA antigen yield of candidate vaccine viruses in eggs

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Candidate vaccine viruses (CVVs) for seasonal influenza vaccines are typically made by classical reassortment in eggs of the vaccine strain with a high yielding egg-adapted strain, PR8, to generate high growth reassortants (HGRs) containing the antigens of the vaccine strain. A high proportion of H1N1pdm09 HGRs contain wild-type (WT) segment 2 when compared with H3N2 HGRs. To understand the molecular basis of this occurrence, we made CVV mimics by reverse genetics that were either 6:2 or 5:3 reassortants between PR8 and two pdm09 strains, differing in whether they contained the pdm09 segment 2 in addition to the glycoprotein genes (antigens). The CVV 5:3 mimics showed greater fitness in MDCK-SIAT cells than the 6:2 viruses. However, Haemagglutinin (HA) antigen yields from eggs of the 6:2 viruses were greater than the 5:3 reassortants. This unexpected phenomenon reflected a temperature sensitivity conferred by WT segment 2, as HA yields from eggs for viruses containing the H1N1pdm segment 2 were ~ 10-20-fold higher at lower temperatures whereas HA yields of the 6:2 viruses were only improved 2-4-fold. The growth of authentic 5:3 HGRs, X-179A and X-181, was not markedly temperature-sensitive however, despite PB1 sequences of all three viruses being identical. This suggests compensatory mutations elsewhere in the HGR genome, the most plausible being in PB2. Overall, these results show that WT segment 2 contains both positive and negative elements for virus growth; a negative element being a temperature-sensitive phenotype. Identification of these elements could result in the ability to manufacture a better growing CVV.

S21/P5

Molecular epidemiology of an outbreak of Lymphocytic Choriomeningitis virus infection in rodents and primates in the United Kingdom.

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Lymphocytic choriomeningitis virus is harboured by house mice and causes diseases in humans and non-human primates (NHPs). Outbreaks of Callitrichid hepatitis (CH) in zoos have been previously reported. In this study we investigated a recent outbreak of CH in a zoo in the UK. Total RNA was extracted tissue samples obtained from 598 rodents and NHPs and transcribed to cDNA. This was used as a template in PCR reactions using published primers targeting a highly conserved region of the Old World arenavirus L-gene. A total of 24 animals (22 mice, 1 black and white colobus and 1 Geoffroy's marmoset) were LCMV positive. Positive samples were sequenced and aligned with published sequences from Genbank. Three sets of degenerate primers and one published set of degenerate primers were then used to amplify the complete LCMV glycoprotein and nucleoprotein which were then aligned with published sequences from Genbank using the Molecular Evolutionary Genetics Analysis (MEGA) Software version 6.06. Alignments showed that the new LCMV strains belong to lineage 1 together with other strains responsible for meningitis and encephalitis in immunocompetent and immunocompromised people and CH in primates. Sequences of positive samples were also aligned with sequences obtained from a similar study carried out in the United Kingdom. The genetic distance between the newly sequenced LCMV and the nearest Liverpool strain was <10% as the LCMV from this study formed a separate cluster. LCMV remains an underreported cause of mortality in infants. Most human cases are not reported because most infections are asymptomatic.

S21/P6

Development of a pseudotype virus-based neutralization assay for phocine influenza virus serosurveillance

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As influenza is an important zoonotic infection, there is considerable interest not only in transmission within normal host populations but also into novel host species including man, as notably illustrated by the 2009 swine influenza pandemic. Marine mammals, such as seals are known to be susceptible to infections by several influenza A subtypes. In recent years there have been large outbreaks of influenza-mediated seal mortality along the US New England and Swedish coasts, killing hundreds of animals. Sequencing revealed the former to be of the H3N8 subtype and latter H10N7, with phylogenetic analysis pointed to an avian origin. In order to establish a tool for serological studies we have developed a pseudotype virus neutralization assay (PVNA). First, we generated replication-defective pseudotype viruses (PVs) using our established plasmid-co-transfection method. Pseudotype particles comprised of an HIV lentivirus 'core' and an envelope containing either the A/harbor seal/Massachusetts/1/2011 H3 subtype or A/chicken/Germany/N49 H10 subtype haemagglutinin (HA) glycoproteins. The PVs were then incorporated into the PVNA, where neutralizing antibody responses are determined by measuring reduction of PV infectivity in target cell cultures. The serum samples utilised were taken from seals caught in the Caspian Sea (CS) at the Europe/Asia border, Baltic Sea (BS) in Southeastern Europe and US Pacific Ocean (PO) coasts. All sera were also tested by competitive ELISA for the influenza nucleoprotein (not subtype specific), using a commercial kit developed at FLI. Data from both PVNAs and ELISA will be presented.

S21/P7

Understanding the host-pathogen dance: Virus-host interactions in an influenza model

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Influenza is a global problem in both humans and livestock, and the virus is continuously evaluating. Therefore, there are high economical loses every year due to inefficiency of available strategies. Epigenetic mechanisms may greatly influence the effectivity of the anti-viral therapies. Understanding of epigenetic mechanisms in host-pathogen systems becomes crucial before designing any disease prediction and prevention strategies. The knowledge may contribute to map the key molecules for defining candidates for biomarker and drug development.

Here, we tested whether the epigenetic status of infected cells are modulated during influenza infection or not. The human A549 alveolar epithelial cell line was infected with H1N1 subtype of influenza. During the course of influenza infections, various histone modifications (acetylation and methylation) were analysed by high-resolution microscopy using fluorescent immunodetection of epigenetic marks. We also determined the global content of DNA methylation using enzymatic approach called the Luminometric Methylation Assay (LUMA). We have extended our analysis to map the infected cell transcriptome to have an overview of the up/down regulated gene profiles involving epigenetic mechanism. The transcriptomic analysis provided us more evidences on the genes involved in epigenetic regulation.

We successfully revealed the influenza infection is accompanied by specific histone modifications in infection models by immunofluorescent microscopy. The LUMA analysis did not show clear DNA hyper/hypomethylation of the genome of H1N1 infected A549 cells . Transcriptomic studies evidenced also a modulation of the expression of genes associated to DNA methylation.

S21/P8

The role of the 627-domain of the PB2 subunit of the influenza A virus polymerase complex

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Avian influenza virus replication is usually severely restricted in mammalian hosts. In order to overcome this species barrier and enable efficient viral replication the avian influenza virus needs to acquire adaptive mutations in its RNA-dependent RNA polymerase. A single amino acid substitution PB2-E627K, which is almost universally present in all human influenza virus strains, is able to overcome this host restrictive effect and enhance avian polymerase activity to the level of mammalian-adapted influenza polymerases. PB2-627 lies on the surface of a structurally distinct domain called the 627-domain, close to the proposed RNA-exit channel of the influenza virus polymerase. Several other adaptive mutations have been described that cluster around the 627-domain and have been shown to act synergistically with E627K or even compensate for the lack it. However, the mechanism of how mutations in the 627-domain enable host adaptation remains unclear. Here we show that the 627-domain is neither required for the assembly of the heterotrimeric polymerase complex, nor for *in vitro* promoter binding, replication initiation and transcription. Nevertheless, in a cellular context we find that the 627-domain remains essential for viral replication, transcription and cRNA stabilisation. These results suggest that the 627-domain does not contribute to the core functions of the polymerase but performs an auxiliary function potentially facilitating the assembly of the newly produced RNA with polymerase.

S21/P10

IL-29 has potential as a novel prophylactic drug against respiratory syncytial virus.

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Respiratory syncytial virus (RSV) is the principal cause of severe lung disease in infants. However, how RSV causes disease in infants is poorly understood. In particular, innate immune responses to RSV infection in human airways, the main target for RSV, are poorly understood. We exploited our well-differentiated primary paediatric bronchial epithelial cell culture model (WD-PBECs) to study IL-29 (IFNλ1) responses to RSV infection. We demonstrated that RSV infection of WD-PBECs induced an antiviral response, mediated in part by secreted factors, capable of significantly reducing Sendai virus growth kinetics. We now show that conditioned basolateral medium from RSV-infected WD-PBECs (CMRSV) also reduces RSV growth. Using an IL-29 neutralising antibody we demonstrated that IL-29 was partly implicated in the antiviral activity associated with CMRSV. Therefore, we assessed the potential of IL-29 as a prophylactic or therapeutic drug against RSV. We found that apical pre-treatment of WD-PBECs with IL-29 significantly attenuated RSV growth kinetics but basal pre-treatment did not. In contrast, neither apical nor basal treatment of WD-PBECs with IL-29 following RSV infection altered virus growth. Interestingly, IL-28RA, a component of the IL-29 receptor, was located exclusively on the apical surface of WD-PBECs, providing an explanation for the polarised efficacy of IL-29 in altering RSV replication kinetics. Apical pre-treatment of WD-PBECs with IL-29 also modulated chemokine responses following RSV infection, as evidenced by decreased IL-6 and MIP-1β/CCL4, and increased IP-10/CXCL10 and MCP-1/CCL2 secretions. Our data suggest that IL-29 has potential as a novel aerosolised prophylactic drug to help combat RSV spread.

S21/P11

Assessing Adhirons against the essential HRSV protein M2-1 and potential new anti-HRSV therapies.

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Human syncytial respiratory virus (HRSV) infects over 64 million people each year across the globe, resulting in over 250,000 deaths. HRSV is the leading cause of lower respiratory tract illness in the immunocompromised, elderly and premature infants. Furthermore, there are prominent links with early childhood infection and asthma in later life.

HRSV is a negative stranded RNA virus, and in order for it to multiply, it must generate a single discrete mRNA from each of the 10 HRSV genes using a multicomponent RNA-dependent RNA polymerase (RdRp) with core components the large (L) protein and the phosphoprotein (P).

Another of essential component is the M2-1 protein, which acts as a transcription antiterminator; without M2-1 short non-functional mRNAs are produced, preventing correct gene expression. Here we describe the generation of M2-1 specific Adhirons, which are non-antibody binding proteins, in order to examine the extent of Adhiron mediated inhibition of M2-1 interactions with viral RNA and P (another essential component of the HRSV polymerase complex). We aim to characterize Adhiron binding sites targeted to M2-1 through the use of biophysical techniques including competition fluorescence anisotropy. X-ray-crystallography will determine the structure of the M2-1-Adhiron co-complex, providing initial pharmacophores at the RNA and P binding site that could direct future drug design. The Adhirons may also inhibit M2-1 in infected cells. Overall, experiments aims to increase understanding of the function of M2-1 and in the long-term provide potential new anti-HRSV therapies.

S21/P13

Investigating the mechanisms by which ANP32 proteins support influenza A virus polymerase function

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Although naturally residing in wild aquatic birds, avian influenza A viruses have been responsible for multiple human pandemics in the last century. Fortunately, pandemics do not occur more frequently due to the existence of host range barriers, which require these viruses to undergo adaptations in order to be able to efficiently infect and transmit between humans. The inability of avian influenza A viruses to replicate efficiently in mammalian cells represents one of the major barriers restricting host range. The most common adaptive mutation which is able to overcome this host restriction is the E627K mutation in the PB2 subunit of the viral polymerase. How this mutation is able to overcome this host restriction however was, until recently, unclear. We now show that that chicken Acidic (Leucine rich) Nuclear Phosphoprotein 32KDa A (chANP32A) is required to support polymerase function of the avian polymerase and that the E627K mutation enables the polymerase to utilise human homologues of this protein to aid in its replicative activity. The mechanisms by which ANP32 proteins facilitate viral polymerase activity are still being elucidated. Using an in situ polymerase assay where cloned virus polymerase proteins amplify and express minigenome reporters, we show that the ability of chANP32A to help activity of the avian polymerase in mammalian cells differs depending on minigenome length. We also start to investigate components of the macromolecular complex involving ANP32 proteins in the context of viral replication.

S21/P14

A mini-replicon and rescue system for Hazara virus

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Mini-replicon and rescue systems are invaluable tools in the study of RNA virus replication as they allow the manipulation of viral genomes (in cDNA format) in order to investigate the function of individual proteins. Hazara virus (HAZV) is a hazard group 2 bunyavirus within the *Nairovirus* genus, and is one of only two members of the Crimean Congo haemorrhagic fever virus (CCHF) serogroup, along with CCHFV. The HAZV genome consists of three segments of negative sense RNA; the small (S), medium (M) and large (L) segments, which encode the viral nucleocapsid protein, glycoproteins and RNA dependent RNA polymerase (RdRp). Here, we describe a mini-replicon system for HAZV, which allows the transcription and replication of HAZV minigenomes consisting of a negative sense reporter gene (EGFP or secretory nanoluciferase) flanked by the HAZV S, M, and L-seg untranslated regions (UTRs). Co-transfection of the HAZV RdRp and nucleocapsid protein with the minigenomes enables their transcription and replication, and subsequent reporter gene expression. We also describe a rescue system, which allows the recovery of infectious HAZV; here the transfection of plasmids encoding the full length HAZV S-, M-, and L-segments into cells results in the re-constitution of infectious virions. The HAZV mini-replicon and rescue systems will enable precise mutations, deletions and insertions to be made within the HAZV genome, which will permit a molecular understanding of HAZV biology through subsequent experiments in comparison to wild-type virus. These systems will also permit the simple and rapid assessment of replication and transcription inhibitors *in vitro*.

S21/P15

EBOLAVIRUS PSEUDOTYPES AS ANTIGEN SURROGATES FOR SEROLOGICAL STUDIES

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The outbreak of Ebola virus disease in West Africa has focussed the global scientific community's efforts on the need for efficacious vaccines and antiviral drugs as well as improved diagnostic assays, all of which should be applicable in the resource-limited countries where Ebolaviruses cause outbreaks. To meet this need we have generated a panel of replication-deficient Ebolavirus pseudotypes using lentiviral cores, optimised the method for their production and identified the most permissive cell lines. We further assessed neutralisation profiles on the three most permissive cell lines (HEK 293T/17, CHO-K1 and CRFK) using a range of monoclonal antibody and serum samples, determining CHO-K1 cells offer the clearest neutralisation data and thus may be more appropriate for use in serology studies, although are less permissive to infection than HEK 293T/17 cells. Comprising the main antigenic target, the Ebolavirus envelope glycoprotein, these pseudotypes alleviate the need for wild-type virus in serological studies and can be handled in a low containment laboratory setting. They have been utilised in a wide range of projects including testing the efficacy

of an Ebolavirus vaccine and the development of novel antibody therapeutics.

S21/P16

Investigating resistance of influenza A virus to a small molecule inhibitor of nuclear export

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Current anti-influenza therapies are hindered by the rapid generation of resistance. The lack of a 'proofreading' function in influenza's polymerase results in a high error rate, allowing the virus to rapidly adapt to certain selection pressures by mutation, thereby generating resistant variants to antivirals such as amantadine and oseltamivir. There is growing interest in the prospect of inhibiting cellular targets, as the generation of resistance is predicted to be slower.

Influenza viruses utilize the cellular nuclear export pathway CRM1 to transport viral ribonucleoproteins (vRNPs) from the nucleus to the cytoplasm of an infected cell, a critical late stage of the influenza lifecycle. Leptomycin B (LMB), a Streptomyces metabolite, has been previously shown to target this pathway, resulting in reduced viral propagation; however LMB's potent cytotoxicity has limited its use as a therapeutic agent. Here we examined a novel small-molecule inhibitor of nuclear export, KPT-335, an analogue of LMB. *In vitro*, we find that KPT-335 inhibits replication of human and animal influenza A virus strains in a dose-dependent manner. To assess the resistance potential of KPT-335, we attempted to generate resistance *in vitro* by serial passage of A/PR/8/34 (H1N1) in the presence of a sub-optimal concentration of the compound. Resistance to KPT-335 took 8-10 rounds of passage to develop. Sequencing and reverse genetics analyses of independently derived resistant virus clones have identified single amino acid changes on a surface exposed loop of NP opposite the RNA binding cleft that confer resistance. The mechanistic basis for this is under investigation.

S21/P17

Defining the cytoskeletal requirements for measles virus-induced cell-cell fusion

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Measles remains a highly prevalent disease in young children especially in the developing world. The absence of specific treatment and low immunization coverage lead to thousands of deaths annually. Measles virus (MeV), the causative agent, is an RNA virus that primarily infects CD150-positive immune cells and Nectin-4-positive cells of the respiratory epithelium. In some cases, MeV also infects the brain where it can develop into severe subacute panencephalitis (SSPE). During infection MeV can induce the fusion of neighbouring uninfected cells in the lymph node, lung and brain to aid dissemination. We have investigated the role of cellular factors in the initiation of virus-induced cell-cell fusion in order to identify potential therapeutic targets. Using a quantitative measles glycoprotein-driven cell-cell fusion assay, we observed that polymerization and branching of cortical actin significantly affects the progression of virus mediated cell-cell fusion. In contrast, microtubule polymerization and the activity of the centrosome contribute to the formation and expansion of the fusion pore. Using a combination of pharmacological inhibitors as well as constructs encoding constitutively active and dominant negative mutants of Rho GTPases, we have shown that cell-cell fusion is greatly affected by the activity of Cdc42 and RhoA, particularly in cells expressing the viral glycoproteins. Finally, we have observed that the expression of MeV glycoproteins leads to the activation of several molecules involved in cytoskeleton rearrangement, including the Ezrin/Radixin/Moesin (ERM) family of proteins and the vasodilator-stimulated phosphoprotein (VASP). Together, our results suggest a MeV-specific modulation of the cytoskeleton that appears to prime cell-cell fusion.

S21/P18

The battle between Rotavirus and the host innate immunity:NSP1-mediated regulation of type I IFN

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Rotavirus (RV) is the most common cause of severe dehydrating diarrhoea, with approximately 590,000 gastroenteritis-associated deaths worldwide in children under the age of 5 years. In the first 24 months of life, almost every child has shown an episode of rotavirus A (RVA) infection. The virus infects both humans and animals but it is usually replication restricted in heterologous mammalians host species, but the underlying reasons for these restrictions are not understood.

Viral infections are recognised by specific host proteins, Pattern Recognition Receptors (PRR). These sentinels sense specific viral components, Pathogen-Associated Molecular Patterns (PAMPs), stimulating through the production of interferons (IFNs) the expression of interferon stimulated genes (ISGs), establishing an antiviral state.

RV non-structural protein 1 (NSP1) exhibits the greatest sequence variability of any of RV protein. NSP1 subverts IFN activation targeting proteins in the IFN induction and signalling pathways in a strain-dependent manner. It has been reported that the bovine strain UK antagonizes IFN expression inducing the degradation of IRF3 and IRF7. However, porcine NSP1 (OSU) seems to target preferentially α-TrCP and RIG-I to block IFN induction.

The aim of my project is to characterize the differential targets of RV NSP1 from different host species, with respect to components of IFN induction and signalling pathways.

Using a yeast two-hybrid assay I will define these targets and I will assess the potential role of NSP1 in driving their proteasome-mediated degradation.

A later "read out" of my project will be the quantification of the overall effect of NSP1 on the expression of IFNs.

S21/P19

Serology testing of H3N2 influenza—correlation of assay outcomes with viral phenotypes and development of improved assays

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Every six months the strains to be included in the seasonal influenza vaccine are selected based on data acquired through serological assays. However, the validity of these data has recently been called into question due to phenotypic changes in viruses of the H3N2 subtype. Recent evidence shows that these viruses have gained the ability to agglutinate red blood cells through their neuraminidase (NA) surface protein, a role previously only carried out by the viral haemagglutinin (HA). This has led to the inclusion of oseltamivir in routinely-used assays so as to eliminate the NA-mediated binding which has been confusing results. While this is effective in isolating the HA-mediated binding, it overlooks any potential physiological relevance of NA and the antibody response to this protein.

A panel of seven recombinant viruses was generated by reverse genetics (RG) which display mismatched HA and NA proteins in order to further investigate their roles in recent H3N2 isolates. These feature the H3 or N2 of a recent isolate, together with either the N1 or H1 of A/Puerto Rico/8/34 (PR8) or the N7 or H7 of A/Equine/Prague/1956. The ability of these viruses to infect cells in the presence of serum raised against the wild-type virus will then provide evidence as to the relative contributions of neutralising antibodies to each of the surface proteins individually. If the antibody response to NA is found to be significant then greater consideration should be paid to its role in vaccines and routine assays designed to assess its relative contribution.

S21/P20

Understanding the Mechanisms by which the Hazara Virus Nucleoprotein Modulates Host Cell Apoptosis

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Hazara virus (HAZV) and Crimean-Congo haemorrhagic fever virus (CCHFV) are members of the *Nairovirus* genus, within the Family *Bunyaviridae*. CCHFV is one of the most lethal human pathogens in existence, causing a fatal outcome in up to 30% of human infections. In contrast, HAZV has not been documented to cause serious human disease and is classified as a biosafety hazard level 2 pathogen. We propose HAZV represents a potentially useful model with which to study nairovirus molecular and cellular biology, and may assist in drug development against the more pathogenic nairoviruses such as CCHFV. The nucleoproteins (NPs) of all negative stranded RNA viruses enwrap and protect their cognate RNA genomes, and in addition recent evidence suggests bunyavirus and arenavirus NPs perform roles in interrupting cellular antiviral mechanisms to enhance viral replication. For CCHFV, there are currently two proposals for how NP delays the host cell antiviral mechanism of apoptosis: either NP distracts caspases with its exposed caspase cleavage sites; or the NP inhibits Bax, which prevents the release of cytochrome c necessary for caspase activation. We assessed the ability of HAZV to interfere with apoptosis, confirming induction at late time points post infection. Informed by our recent determination of the CCHFV and HAZV NP crystal structures, we investigated the role of caspase-3 motifs in apoptosis delay by expressing NP proteins with altered caspase cleavage properties, and monitoring apoptosis induction. In addition, the role of NP in altering mitochondrial membrane potential was investigated to offer insight into whether NP modulates Bax.

S22/P1

Regulation of production of gas vesicles, motility and carbapenem and prodigiosin antibiotics in *Serratia*.

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Serratia sp. ATCC 39006 (S39006) is the only member of the Enterobacteriaceae reported, to our knowledge, to produce gas vesicles (GV) naturally, enabling bacterial flotation in aqueous environments. GVs are hollow intracellular organelles produced in S39006 cultures at high cellular densities and their production is under both quorum sensing (QS) and aeration control. In order to identify other regulatory inputs to GV production, we performed random transposon mutagenesis. 32,432 insertion mutants were screened and 27 strains exhibiting altered GV production were identified, followed by sequencing and bioinfomatic analysis. Two new regulators of GV production were identified and characterised; a low affinity potassium transporter (TrkH) and the glycerol 3-phosphate regulon repressor, GlpR. We found that mutations in both of the corresponding genes showed phenotypic impacts on flagellar motility and production of the prodigiosin antibiotic, whereas production of the carbapenem antibiotic and the QS signalling molecule, N-butanoyl-L-homoserine lactone (BHL) was affected only in the *glpR* background. Our findings imply that potassium levels and glycerol availability may be environmental or metabolic cue inputs for GV morphogenesis, motility and bioactive secondary metabolite production in S39006.

S22/P2

Social behaviour in motile colonies of *Staphylococcus aureus*

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It has been shown that *Staphylococcus aureus* can move across surfaces via two behaviours: 1. passively moving across the surface of agar plates in a process called spreading (a form of sliding), and 2. the formation of dendrites by 'comets' of cells that move in a manner that share many similarities with a form of active motility known as gliding. Both behaviours are dependent on surfactant production, which is regulated by the *agr* locus, the quorum sensing system in *S. aureus*.

Agr also regulates the production of secreted virulence factors and the mediation of this has been previously shown to be a social trait that can be exploited by *agr* mutants. We therefore investigated whether *S. aureus* motility could also be an exploitable social trait as it also relies on *agr* regulated secreted exoproducts (the surfactants in this case). We experimentally tested this by mixing together Newman wild type and an *agr* mutant and measuring overall spread of the colony, total cfu counts and the proportions of each throughout spreading colonies. We found that contrary to what we predicted the agr mutant did not have a deleterious effect on the spread of the WT and they both benefitted from each other's presence. This indicates some synergistic interaction may be taking place and the type of social interaction that *agr* mediates is context dependent.

S23/P1

Comprehensive scrutiny of various factors that crucially govern the codon and amino acid usage patterns in the genus Bifidobacterium.

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Members of the genus Bifidobacterium are essential residents of the human, animal and insect gut, associated with beneficial probiotic activities. An extensive investigation on codon and amino acid usage of the GC rich genus Bifidobacterium has been executed in the present study. It was evident from multivariate statistical analysis that a coupled effect of GC compositional constraint and natural selection for translational efficiency was operative in producing the observed codon usage variations. Gene expression level was inferred to be the most vital factor in shaping the mode of codon usage. Amino acid usage was found to be considerably influenced by hydrophobic and aromatic character of the encoded proteins. Gene expressivity and protein energetic cost also had substantial impact on the differential mode of amino acid usage. The genus was found to strictly comply with the cost-minimization hypothesis as was reflected from the amino acid usage patterns of the potential highly expressed gene products. Evolutionary analysis confirmed that the highly expressed genes were candidates to extreme evolutionary constraints and indicated a high degree of conservation at the proteomic level. Interestingly, the complimentary strands of replication appeared to evolve under similar selection pressure which might be addressed as an upshot of absence of replicational selection and lack of strand-specific asymmetry among the members of the genus. Thus, the present venture provides considerable information pertaining to the riddles of codon and amino acid usage in Bifidobacterium and might prove effective for further scientific progress associated with the concerned domain of research.

S23/P2

Investigating how the regulation and chromosomal location of the growth-phase specific nucleoid associated proteins, Fis and Dps, affect bacterial fitness in Salmonella Typhimurium.

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Nucleoid associated proteins (NAPs) are global gene regulators and the differential expression of NAPs is responsible for the fitness of bacteria at various growth phases and under different growth conditions. Growth phase specific NAPs are those which are highly abundant only within one growth phase. In Salmonella these growth-phase specific NAPs include Factor for Inversion Stimulation (Fis) and DNA-binding protein from starved cells (Dps). Fis expression is maximal at early exponential phase and is the most abundant NAP during this growth phase. Dps expression increases in early stationary phase and Dps becomes the most dominant protein during this time. Understanding the differential regulation of NAPs and their effects on global gene regulation is essential for synthetic biology. As the field of synthetic biology further develops, and as more complex biological systems are designed and re-designed, it is necessary to consider how even the locus of a NAP can affect surrounding genes, further downstream pathways and the overall fitness of the biological system. Research in this area may contribute to the creation of microbes which can be used in industrial processes. In this study we are investigating the effects of swapping the chromosomal locations of fis and dps genes on the fitness and physiology of Salmonella Typhimurium. Separately, we also aim to investigate the growth-phase regulation of these two NAPs by swapping the fis and dps open reading frames.

S23/P3

Epistatic Interactions in the Arabinose *cis*-Regulatory Element

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Changes in gene expression are an important mode of evolution, however the proximate mechanism of these changes is poorly understood. In particular, little is known about effects of mutations within *cis* binding sites for transcription factors, or the nature of epistatic interactions between these mutations. Here, we tested the effects of single and double mutants in two cis binding sites involved in the transcriptional regulation of the *Escherichia coli araBAD* operon, a component of arabinose metabolism, using a synthetic system. This system decouples transcriptional control from any post-translational effects on fitness, allowing a precise estimate of the effect of single and double mutations, and hence epistasis, on gene expression. We

found that epistatic interactions between mutations in the *araBAD cis* regulatory element are common, and that the predominant form of epistasis is negative. The magnitude of the interactions depended on whether the mutations are located in the same or in different operator sites. Importantly, these epistatic interactions were dependent on the presence of arabinose, a native inducer of the *araBAD* operon *in vivo*, with some interactions changing in sign (e.g., from negative to positive) in its presence. This study thus reveals that mutations in even relatively simple *cis* regulatory elements interact in complex ways such that selection on the level of gene expression in one environment might perturb regulation in the other environment in an unpredictable and uncorrelated manner.

S23/P4

Regulation of biofilm formation in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen that dominates the airways of cystic fibrosis (CF) patients. Chronic *P. aeruginosa* infection is strongly linked with an increased risk of respiratory failure and death. *P. aeruginosa* adopts one of two lifestyles; growth in "free living" or planktonic cultures, or as sessile biofilms. The latter are strongly associated with chronic infection and are characterised by the formation of aggregated clumps of bacteria embedded within a unique self-secreted exopolysaccharide matrix. This matrix acts as a protective barrier against anti-microbial agents and also aids immune evasion, thus making chronic infections very difficult to eradicate. Currently there is limited understanding as to what genetic factors promote the biofilm mode of growth. The aim of this project is to identify such regulatory factors. Using stable chromosomal reporter constructs to monitor expression of the biofilm associated genes *pslA* and *cdrA*, mutations are being randomly introduced into the genome using plasposon mutagenesis. Insertions that alter *cdrA* or *pslA* expression may result from integration of the plasposon into regulators specific to either *pslA* or *cdrA*. Alternatively, they may "hit" a global biofilm regulator. Disrupted genes will be identified and mutants will be characterised further to identify defects in biofilm formation and exopolysaccharide production. Microarray analysis will help to uncover the regulatory network driving biofilm formation and may highlight potential environmental triggers that activate the transition from planktonic growth into this highly resistant biofilm mode of growth.

S23/P5

Presence of a chlorite dismutase gene in an IncP-1 plasmid present in *Pseudomonas aeruginosa* 943 may influence biofilm formation and chlorite resistance in chlorine dioxide treated hospital water supply

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Pseudomonas aeruginosa is a nosocomial bacterial pathogen which causes secondary infections in vulnerable individuals, particularly in hospital Burns Units. Most strains have the capacity to form biofilms and disseminate antibiotic resistance genes to other strains via horizontal gene transfer, making treatment difficult. This study investigates the isolation of *Pseudomonas aeruginosa* 943 carrying aplasmid called pBURNS1 which carries a *cdm* gene encoding a putative chlorite dismutase. The strain was isolated from both water and shower environment samples in the Burns Unit of the new Queen Elizabeth Hospital Birmingham indicating that it may have originated from an established biofilm. It is hypothesized that selective pressure from chlorite, which may have arisen from the chlorine dioxide treatment of the hospital water or from treatment further upstream, influenced the survival of these strains within the hospital. The MIC, MBC and MBEC of the plasmid positive and negative strains, differences in biofilm formation and survival in both strains in planktonic and resuspended biofilm states were conducted in the presence of chlorine dioxide and chlorite solutions to test this hypothesis. Knowledge of the origin of the strain and the influence of plasmid-encoded genes in surviving water treatment supply will be useful to environmental and public health in combating plasmid borne antibiotic and biocide resistance.

S23/P6

Population Genomics of *Campylobacter spp.*

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Campylobacter spp. is the leading cause of infectious intestinal disease in several developed countries worldwide, including the United Kingdom. It's highly associated with the consumption of poultry, which is attributed to ~70% of all occurrences. During 2012, in England and Wales alone there were more than 65,000 laboratory confirmed cases, leading to a projected 15,000 hospitalizations and 76 deaths. Estimates indicate that a further

7.6 infections occur for every case that is reported, bringing the approximate number closer to 500,000. *Campylobacter* is responsible for a third of all expenditure on food-related illness, and costs the UK economy of upwards of £580 million each year.

The organism itself is unremarkable, and you'd be forgiven for thinking it to be innocuous at first glance. But the reality is that this humble yet tenacious bug continues to elude our best attempts at preventing entry and persistence in our food chain.

In this work, we take a look at *Campylobacter* across the greater Nottinghamshire area. By whole genome sequencing clinical isolates obtained from Queens Medical Centre – who turnover 800+ confirmed cases a year – we have constructed a key insight into how the *Campylobacter* population is structured, and how this compares to other parts of the United Kingdom.

Additionally, we undertake a novel approach to genome sequencing by looking at an infection as a population of individuals and analyse the presence of minor allele variations across a single infection.

S23/P7

Defining the population structure of *Escherichia coli* in the environment

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) have the ability to cause a diverse range of serious diseases, such as urinary tract infections (UTIs) and bacteraemia. Many of the most important global strains of human ExPEC, such as the multidrug resistant (MDR) *E. coli* sequence type (ST) 131, are well characterised in clinical cases, such as UTIs. The high clinical prevalence of such strains would imply a wide range of potential sources other than the human intestinal tract, which would include non-human reservoirs such as retail meat products, agricultural runoff, sewage wastewater, companion animals, and other environmental sources. Studies conducted to date have not adequately characterised the population structure of *E. coli* in the wider environment. In this work, we carry out a study to define the population structure of *E. coli* from a range of environmental samples that represent potential non-human reservoirs for ExPEC. Our approach, which involves whole genome sequencing of environmental isolates, allows us to construct a snapshot of the prevalence of MDR *E. coli* in the wider environmental *E. coli* population and how this compares with the population structure of clinical isolates.

S23/P8

Investigating the importance of the chromosomal positioning of the genes encoding the nucleoid associated proteins, H NS and StpA, in *Salmonella enterica* serovar Typhimurium

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The carefully coordinated and timed expression of nucleoid associated proteins (NAPs) is important for governing global gene expression and nucleoid architecture in bacteria. Furthermore, it is now recognised that the chromosomal positioning of a gene has an impact on the expression profile of that gene. A recently published study from our laboratory demonstrated that reciprocally swapping the chromosomal locations of the paralogous *hns* and *stpA* ORFs in *Salmonella* Typhimurium resulted in a strain with superior competitive fitness to that of wild type *S.* Typhimurium (1). In the present study we are building upon these novel findings by performing a reciprocal exchange of the entire *hns* and *stpA* genes, including their upstream regulatory regions. The *hns* gene is located near the terminus of chromosome replication and H-NS levels remain high throughout bacterial growth, while the *stpA* gene is located approximately mid way between the origin and terminus of chromosome replication and StpA is detected mainly during exponential phase growth. We are investigating how chromosomal location contributes to the distinct expression pattern of the *hns* and *stpA* genes; and how rewiring the expression pattern of these important NAPs affects bacterial fitness, stress resistance, gene expression and DNA topology. Detailed analyses of these genetic regulatory circuits may contribute to the rational design of engineered microbes for use in synthetic biology and biotechnological processes.

1. Fitzgerald S, *et al*. (2015) Re-engineering cellular physiology by rewiring high-level global regulatory genes. *Scientific Reports* 5:17653.

S23/P9

The Mouse Intestinal Bacterial Collection (miBC): Host-Specific Insight into Cultivable Diversity and Genomic Novelty of the Mouse Gut Microbiome

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Molecular techniques have generated major breakthroughs in microbial ecology of the mammalian gut. However, it became clear in recent years that isolation, thorough characterization and archiving of gut bacteria is urgently needed for description of novel diversity, for amendment of genomic databases, and for the sake of gnotobiotic studies. By establishing the mouse intestinal Bacterial Collection (miBC), we provide the first state-of-the-art repository of bacterial strains and associated genomes from the mouse intestine. Approximately 1,500 aerobic and strictly anaerobic isolates were obtained from various gut location, mouse strains and facilities. We selected 100 bacteria representing 76 species across 26 families that cover the majority of known phylogenetic diversity in the mouse gut. A total of 18 species found to be most prevalent or enriched in the mouse intestine were identified. Genome sequences from these and additional collection members were obtained, providing new mouse-derived bacterial genomic information (53 draft genomes). Novel diversity was also described via taxonomic characterization of 15 new bacteria, including a species of the so far uncultured S24-7 family within the Bacteroidales as well as novel butyrate producers. Genomic analyses showed that certain species are specific to the mouse intestine, and that a minimal consortium of 18 strains covered 50 to 80 % metagenomic functions. The present study is an important foundation for future research on diet-microbiome-host interactions in health and diseases as it will facilitate molecular and targeted colonization studies.

S23/P10

Bioinformatics analyses of genetic variation in genomes of *Neisseria meningitidis* (the meningococcus)

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While *Nessieria meningitdis* can be one of the most harmful pathogenic bacteria this species usually colonizes hosts without causing disease. Genomic variation of *N. meningitdis* is one of the mechanisms that enable meningococci to survive host defense strategies. An analysis was performed of genic and intergenic variation for 40 isolates that were isolated as 10 replicates from four different time points. In the 40 isolates, the intergenic variation was compared with genic variation. Firstly, dynamic of variation was analysed thereby most of the variable genes varied by a single point mutation in genic and intergenic region. In addition, the number of variable intergenic region was 18 in genic and 20 in intergenic regions. Secondly, the mean of nucleotide diversity using the McNemar test showed a significant correlation between variations with time point for genic region but no significant correlation with intergenic region. Thirdly, the positions of mutations in genic regions were seemed likely to be important for adaptation of *N. meningitidis* for some variable loci. However, intergenic variation of the 40 isolates showed that the variation was entirely due to differences in repetitive-DNA sequences. Corriea elements varied within promoter regions and IHF binding sites that have a role in changing expression of genes and exhibited a significant correlation between variation and time point. We speculate that Corriea elements have a role in adaptation to stress condition. Conversely, variation in NIME showed no significant correlation with time point. Similarly, REP2 did not show any variation between different isolates

S23/P11

Title: Effects of inactivation of the *dnaK* chaperon gene on *Clostridium difficile*

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Clostridium difficile is a Gram-positive, anaerobic, sporulating nosocomial pathogen sometimes carried in the human gut as a harmless commensal organism. Exposure to antibiotic therapy disrupts the normal colonic microflora, and allows *C. difficile* to proliferate, whereupon it expresses the main virulence factors - toxins A and B - to establish *C. difficile* infection (CDI). Our main objective was to assess the phenotypic differences between *C. difficile 630*, *C. difficile 630Δ erm*, and two ClosTron generated mutants: one, in the master regulator of sporulation, *Spo0A* (Heap et al, 2009), and the second, in the HSP 70 chaperone gene, *dnaK*. *C. difficile 630-Δerm::dnaK* grew more slowly than the other strains at 37°C, and exhibited a relatively filamentous phenotype with an average cell length 12 µm compared to the other strains (4.X µm) (p=0.001). *C. difficile 630-Δerm::dnaK* mutant was less motile when compared with other strains at 24, 48 and 72h. Factors affecting lowered motility of *C. difficile 630-Δerm::dnaK* were investigated by indirect immunofluorescence for detection of the FliC (flagellin) protein on cell surfaces: microscopy revealed that FliC was not present on the surface of *C. difficile 630-Δerm::dnaK* mutant cells, but was present on the other strains. This indicates that FliC (Flagellin) is not present on the surface of *C. difficile 630-Δerm::dnaK* mutant. We have also shown that *C. difficile 630-Δerm::dnaK* forms a more extensive biofilm, more rapidly, than that observed in the other strains.

S23/P12

PLASMIDS OF THE SALTRESITANT STRAINS OF MICROORGANISMS ARE THE MAIN COMPONENTS OF THE NEW GENERATION OF MICROBIAL FERTILIZER *Murodova S.S National University of Uzbekistan named after MirzoUlugbek Tashkent 100174, Uzbekistan.*

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It is well known that plasmids define the main cell functions such as fertilization, virulence, biodegradations, synthesis of antibiotics as well as resistance to antibiotics, heavy metals and bacteriophages. Presence of a plasmid in thecell can lead to dilating of a range accessible to a cell of nutrients. In our work the attention was devoted to studying plasmid DNA of the salt resisting strains of microorganisms which are a component of a newgeneration of microbial fertilizers. During studying the DNAplasmid of microorganisms'strains such as *Bacillus subtilis - 309*, *Bacillus megaterium -310*, P*seudomonas stutzeri -308* there have been revealed some large plasmids. The electrophoretic analysis, have been revealed plasmid DNA The electrophoretic analysisof the researched plasmides showed the plasmides molecularweight lies in the dimensions 48,5; 30, 13,3 (*Bacillus subtilis*), 23,1 (*Bacillus megaterium*) and 55 kb (*Pseudomonas stutzeri -308*).We suggest thatthese large plasmids might bear genes of stability to unfavorable environmental conditions. The strains of microorganisms appliedasthenew microbiological fertilizerare tolerant to the high concentration of toxic salts containing Cl - and $SO4^{2}$ ions.

S23/P13

Distribution of integrons and gene cassettes among uropathogenic and *diarrheagenic Escherichia* coli isolates in Iran

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Integrons are considered to play a significant role in the evolution and spread of antimicrobial resistance genes. A total of 200 uropathogenic (UPEC) and diarrheagenic *Escherichia coli* (DEC) isolates from outpatients were investigated for antimicrobial susceptibility and the presence of class 1, 2 and 3 integron-associated integrase (*intI*) genes and gene cassettes. Conjugal transfer and Southern hybridization were performed to determine the genetic localization of class 1 integrons. One hundred and ninety two (96%) isolates were resistant to one or more antimicrobial agents. Antimicrobial resistance among DEC isolates was higher than that of the UPEC. Integrons were highly prevalent in both pathotype (92.5%). Comparision of integrons among UPEC and DEC showed that DEC isolates harbored integrases (94% for *intI1*, 8% for *intI2*) with slightly higher frequency than in UPEC isolates (87% for *intI1*, 7% for *intI2*) (P> 0.05). Dihydrofolate reductase (*dfrA*) and aminoglycoside adenyl transferase (*aad*) gene cassettes were found most frequently in *intI1* positive isolates. All isolates carried their class 1 integrons on conjugative plasmids. These results indicate that class 1 integrons are widespread among *E. coli* isolates. Therefore, appropriate surveillance and control measures are essential to prevent the further spread of integron producing isolates.¬¬

S23/P15

Coordinate regulation of antimycin and candicidin biosynthesis

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Antimycins are made by *Streptomyces* bacteria and inhibit the terminal step in electron transport. They have potent bioactivity against a range of organisms and are selective inhibitors of the Bcl-2/Bcl-xL-family of anti-apoptotic proteins which are over-produced in drug-resistant cancers. Antimycins are produced by a hybrid non-ribosomal peptide synthetase / polyketide synthase biosynthetic pathway which we identified and characterised in *Streptomyces albus* S4. The biosynthetic gene cluster consists of 15 genes organised into three operons: *antBA*, *antCDE*, *antFG* and *antHIJKLMNO*. The gene cluster harbours a single cluster-situated regulator, an orphan alternative RNA polymerase sigma factor encoded by *antA* (σAntA). σAntA regulates the expression of *antFG* and *antHIJKLMNO*, which direct the biosynthesis and activation of the 3-formamidosalicylate precursor, but not *antBA* or *antCDE*. Here we show that antBA and antCDE are regulated by FscRI, a LuxR-family cluster-situated regulator of the polyene antifungal agent, candicidin. We also report cloning of the antimycin biosynthetic pathway and demonstrate production of antimycins by a heterologous host is strictly dependent on co-expression of FscRI. These data show there is crosstalk between cluster-situated regulator proteins and that *S. albus* S4 coordinately regulates the expression antimycin and candicidin, suggesting that these two antifungals may act synergistically.

S23/P16

Peptidoglycan assembly and β-lactam resistance in MRSA

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Methicillin resistance in MRSA is mediated by an acquired mecA gene encodes a methicillin-insensitive transpeptidase called penicillin binding protein 2A (PBP2A) which has extremely low affinity for nearly all α-lactams. PBP2A takes over transpeptidase activity of native enzymes and continue cell wall peptidoglycan biosynthesis. While mecA is a prerequisite for expression of resistance, in some clinical isolates only a subpopulation of cells express high level resistance (heterogeneous resistance), while in some isolates the whole population exhibit high level resistance (homogeneous resistance). This heterogeneously resistant isolates can be converted to highly resistant isolate upon exposure to elevated levels of antibiotics. This conversion is regulated by efficient production of PBP2A and chromosomal mutation(s). To investigate underlying mechanism of high level resistance to α-lactams and peptidoglycan biosynthesis, we constructed MSSA-MRSA in a genetically defined background by introducing mecA into methicillin sensitive isolate, while exposure to elevated level of antibiotic selected mutants expressing range of phenotypic resistance pattern from 1 µg/ml to ≥256 µg/ml of oxacillin mic. The whole-genome sequencing approach revealed compensatory mutation within gdpP (GGDEF domain protein containing phosphodiesterase) gene in all cherry picked highly resistant mutants. The highly resistant mutant regained sensitivity upon curing mecA. Reintroduction of mecA into cured background exhibited immediate high level resistance suggesting the role of chromosomal mutation. These genetically amenable strains are being used to construct fluorescent PBP2A for further localisation studies.

S23/P17

Molecular characterization of *Salmonella* isolates: Efficacy of the partial 16S rRNA gene sequencing for the epidemiological tracing of sources of *Salmonellae* during epidemics.

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Serotyping of *Salmonellae* for epidemiological tracing has been deemed inefficient. This study was designed to explore the efficacy of coupling the 16S rRNA gene sequencing with the standard laboratory methods for the precise epidemiological tracing of *Salmonellae* during epidemics. We isolated *Salmonellae* from geographically distant *Salmonella*-infected humans, camels, and poultry. We conducted conventional serological, microbiological and biochemical tests on the isolates, and initially identified them as *Salmonella* strains S-117, S-118 and S-119. We determined the phylogenetic affiliation of the three *Salmonella* isolates by conducting a partial sequencing of their 16S rRNA genes and compared them to sequences of representative organisms belonging to *Enterobacteriaceae* using the alignment editor ae2. We found that the *Salmonella* isolates S-117, S-118 and S-119 share identical 16S rRNA gene sequences and analogous GC content, indicative of their tight phylogenetic cluster. The isolates also shared 100% partial 16S rRNA gene sequence identity with *S. typhi* ATCC 19430T, but divergent H2S production pattern. A phylogenetic dendrogram analysis unvielled that the three *Salmonella* isolates are homogeneous species of *S. dublin*. This finding demonstrates the reliability of the partial 16S rRNA gene sequencing coupled with the standard laboratory testing as an efficient epidemiological tracing tool for *Salmonellae* identification during epidemics.

Keywords: *Salmonella*, partial 16S rRNA gene sequence, epidemiology.

S23/P18

Virulence characterization of Campylobacter jejuni isolated from resident wild birds in Tokachi area, Japan.

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The prevalence of *Campylobacter jejuni* in wild birds is a potential hazard for human and animal health. The aim of this study was to establish the prevalence of *C. jejuni* in wild birds in Tokachi area, Hokkaido, Japan and investigate their virulence *in vitro*. In total, 173 cloacal swabs from individual wild birds were collected for the detection of *Campylobacter* spp. Thirty four samples (19.7%) were positive for *Campylobacter* of which 94.1% (32/34 samples) were *C. jejuni*. Additionally, one *C. coli* and one *C. fetus* were isolated. Seven *C. jejuni* isolates (one from crows and the other from pigeons) had important virulence genes including all three CDT genes (*cdtA*, *cdtB* and *cdtC*) and *flaA*, *flaB*, *ciaB* and *cadF*, and the other isolates were lacking *cdtA* gene. Further studies on *in vitro* virulence-associated phenotypes, such as motility assay on soft agar and invasion assay in Caco-2 cells, were performed. The wild bird *C. jejuni* isolates adhered and invaded human cells. Although the numbers of viable intracellular bacteria of wild bird isolates were lower than a type strain NCTC11168, they persisted at 48-hr and underwent replication in host cells.

S23/P19

Whole-Genome Sequencing to Investigate Antibiotic Resistance and the Molecular Epidemiology of Clinical Isolates of *Brachyspira hyodysenteriae* from the North East of England.

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Brachyspira hyodysenteriae is a Gram-negative spirochaete found in pigs throughout the world. It is the causative agent of swine dysentery, an intestinal disease of pigs characterised by bloody mucoid diarrhoea and poor growth. This disease causes serious economic loss to the pig industry. The pleuromutilin antibiotics, tiamulin and valnemulin, remain a key part of effective treatment and control. However, increased incidence of pleuromutilin resistance has been reported in some countries. This can result in fewer therapeutic options being available to treat *B. hyodysenteriae*, due to increasing chance of infections with multidrug resistant *B. hyodysenteriae*. In England, the diagnostic rate has reduced in recent years but the prevalence of infected herds is not known; outbreaks on commercial units are mostly captured in GB disease surveillance data but herds with endemic infection may not be. The prevalence of tiamulin resistance is also not known and the population structure of *B. hyodysenteriae* has not been investigated. The Animal and Plant Health Agency (APHA) archives isolates of *B. hyodysenteriae* from pig diagnostic submissions from England. We have genome-sequenced a panel of isolates from North-East England, one of the main pig-producing areas of the UK. We have compared the putative resistance genotype to phenotype, identified using VetMic Brachy plates. In addition, we performed an in-depth analysis of the phylogeny of clinical isolates from this region, over a 10-year period to identify circulating clones.

S23/P19

The distribution of prophages in UK and African populations of ST313 Salmonella Typhimurium

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In the past 30 years, Salmonella bloodstream infection has become a significant health problem in sub-Saharan Africa, and is now killing ~390,000 people each year. Infections are predominantly caused by Salmonella enterica serovar Typhimurium, and whole genome sequencing of invasive disease isolates has shown that most strains belong to the novel sequence type ST313 and cluster into two distinct lineages.

Until recently, Salmonella ST313 had only been identified in sub-Saharan Africa. The new routine sequencing of clinical isolates by the Public Health England Salmonella Reference Service has revealed that S. Typhimurium ST313 bacteria also cause disease in the UK. Genomic analysis shows that the UK-ST313 isolates represent phylogenetically intermediate lineages between the two African lineages.

The prophage content of the UK ST313 isolates was compared to the African strains. Despite not being phylogenetic neighbours, both the African ST313 lineages contain two novel prophages, which are not present in UK strains. The parallel accessory genome between these two African lineages implies a high selection pressure for the prophages in the African ST313 niche. These findings suggest that the acquisition of novel prophages may have played a role in the evolution of the African lineages of S. Typhimurium ST313.

S23/P19

ETT2 revisited: Evolutionary dynamics of the second *E. coli* type III secretion system locus

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The *E.coli* type three secretion system 2 (ETT2) is a 29.9kb genomic island present in enterohaemorrhagic E.coli strain O157:H7 EDL933 and enteroaggregative *E. coli* strain 042. The island, which is distinct from the well-characterised LEE pathogenicity island found in enterohaemorrhagic and enteropathogenic *E. coli*, consists of 35 genes, including some homologues of genes encoding the Salmonella enterica SPI-1 type three secretion system. A previous PCR-based study of the ECOR collection of phylogenetically diverse *E. coli* strains found ETT2 to be intact in a minority of strains but present as a partial remnant in most *E. coli* genomes, although it was absent from phylogenetic group B2. It was concluded that the island had been acquired by the other *E. coli* phylogenetic groups after the divergence of group B2 strains, and subsequently been partially deleted in most lineages. Here we examine this conclusion by investigating the distribution of ETT2 and eip in a collection of over 2000 sequenced *E. coli* genomes. A full-length ETT2 island was identified in the C-I clade of *E. coli* strains which fall outside the established diversity of *E. coli* as represented by the ECOR collection. This C-I ETT2 is similar to the ETT2 found in group D1 strains such as 042, suggesting that the island may have been the subject of a recombination event between *E. coli* lineages.

S24/P1

Doxycycline-treated *Wolbachia* retain infectivity in mosquito cells

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Wolbachia pipientis (order *Rickettsiales*) is an obligate intracellular bacterial symbiont of arthropods and parasitic nematodes, including filariae of public health importance. Antibiotic therapy targeting *Wolbachia* as alternative or adjunctive treatment of lymphatic filariasis and onchocerciasis is an active research area.

Tetracycline antibiotics are filaricidal, but the lengthy and sustained treatments required are not practicable for mass administration. Recent work on *Onchocerca ochengi* in cattle demonstrated that for a given dose, intermittent administration over a longer period is more effective than a single continuous treatment.

Using an *in vitro* mosquito cell model, we investigated the effect of treatment regimen on *Wolbachia* survival and ability to transfect naïve cells. *Aedes albopictus* mosquito cells infected with *Drosophila*-derived *Wolbachia* were treated with doxycycline for 7 days, with or without a 7-day period of drug-withdrawal, and treated cells transfected into populations of naïve mosquito cells. Real-time PCR of 16S (bacterial) and 18S (host cell) rRNA genes and immunofluorescence techniques were used to investigate *Wolbachia* growth dynamics in the transfected cells. We demonstrate for the first time that doxycycline-treated *Wolbachia* can re-establish and sustain infection in naïve cells for a minimum 6-week period, with and without post-treatment drug withdrawal. Post-transfection bacterial densities were lower in treated inocula compared to untreated positive controls. Initial *Wolbachia* infectivity was significantly more suppressed immediately after drug-treatment (P <0.05) than after post-treatment drug withdrawal, but the new infection in naïve cells appeared stable in either case. These findings support further research into regimen optimisation and mechanisms of antibiotic tolerance in *Wolbachia.*

S24/P2

Virulence and pathogen adaptation following host shifts in an insect-virus model

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Emerging infectious diseases are often the result of a host shift, where the pathogen originates from a different host species. Virulence - the harm a pathogen does to its host - can be extremely high following a host shift (for example Ebola, HIV, and SARs), while other host shifts may go undetected as they cause few symptoms in the new host. For a host shift to be successful, pathogens may then need to adapt to the novel host. We have examined how virulence varies across host species by carrying out a large cross infection experiment using 48 species of Drosophilidae and an RNA virus. Host shifts resulted in dramatic variation in virulence, with benign infections in some species and rapid death in others. To examine how viruses evolve in new hosts, we then experimentally evolved viruses in 19 of these host species to look for phylogenetic signals of adaption at the molecular and phenotypic levels.

S24/P3

Optimization of miRNA extraction from insect infected with M. anisopliae

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Entomopathogenic fungi, such as Beauveria bassiana (Bals.) and Metarhizium anisopliae (Met.) Sorokin, whose natural habitat is soil, have been found to infect a wide range of insect species (Toledo et al. 2006). It is important to understand the genetic mechanisms which define host range and, therefore aggressiveness of the fungal pathogens. There is a need to reduce the dependence on chemical pesticides and develop an understanding which can lead to pathogens targeted to specific pests. The discovery of miRNAs in the M. anisopliae suggests early origins as a gene regulator (Zhao et al. 2007, Zhou et al. 2012). MicroRNA could play a significant role in development or growth through modulation of translation and mRNA stability of the target genes (Bartel, 2009). This work aims to identify, quantify, and determine whether microRNA play a role in the regulation of insect infection by the entomopathogenic- fungus M. anisopliae. In addition, to determine what genes are targeted by miRNA regulation to better understand their function in fungal development during infection. The experiments to date have focused on developing the methodology for extracting miRNA and total RNA from the Metarhizium infection process. In conclusion, we have compared three commercial miRNA isolation kits for the best performance in extracting miRNA from insects infected with M. anispoliae.

S24/P4

Xenosurvelliance and pathogen detection in British mosquitoes (Diptera: Culicidae)

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The detection of infectious organisms in the blood meal of arthropods provides an insight into the pathogens infecting the host without the need for direct intervention. We have applied this approach, termed xenosurveillance, to the blood-meals of mosquitoes collected in the southeast of England, an area at risk

of potential introduction of zoonotic arthropod-borne viruses. We have tested blood meals in a range of morphospecies commonly found in the study area, for example Anopheles maculipennis complex, Culex pipiens, Culiseta annulata, and Ochlerotatus detritus. In addition to identifying a broad range of vertebrate hosts as the source of the blood meals, we conducted specific PCR for the detection of DNA viruses and also screened for the presence of piroplasms. We found specimens of An. atroparvus (a member of the Maculipennis complex) were positive for Myxoma virus, which suggests that this species may mechanically transmit this virus and lead to the dissemination of myxomatosis. Moreover, we found specimens of Cs. annulata that had fed exclusively on cattle, were positive for Theileria orientalis complex. This Theileria is pathogenic for ruminants in Asia and Australia, although does not appear to cause overt disease in the United Kingdom. We also performed specific PCR for Avian pox and Plasmodium-like lineages on blood-fed specimens of Cx. pipiens, although these were negative. Our approach shows that it is possible to screen for pathogens, vertebrate host and also delineate the species from the DNA extract of a fully engorged female.

S24/P6

A frosty reception for *Spiroplasma:* temperature impacts on an insect-microbe symbiosis

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Most insect species carry vertically-transmitted endosymbiotic bacteria, which persist either by manipulating or by forming mutualisms with their hosts. Host-symbiont interactions are frequently studied under standard 'ideal' conditions. However, the natural environment is commonly variable and harsh, and thus the evolutionary ecology of bacterial symbionts is poorly understood. We investigated the impact of thermal environment on the interaction between *Drosophila hydei* and its protective symbiont, *Spiroplasma* strain hy1. Contrary to earlier work on this system, we observed that transmission was relatively robust to cold temperatures, indicating the potential for symbiont persistence in much of the host's temperate range. These results highlight the importance of selecting ecologically-relevant temperatures in future investigations of symbiont transmission and phenotype.

S24/P7

'Friend or foe: Establishing the role of a symbiont in honey bee health'

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Honey bee populations have witnessed significant declines over the last century. This is a concerning trend, as bees represent major crop pollinators and provide economically important products. A symbiotic bacterium, of the genus Arsenophonus, has recently been identified in honey bee colonies expressing poor performance. Arsenophonus species are commonly associated with a variety of insect hosts and are capable of diverse symbioses, ranging from parasitic son-killers to coevolving mutualists. However, the Arsenophonus sp. - honey bee interaction remains uncharacterised despite a potential role in colony health. To investigate this interaction, we are screening colonies across the UK to determine the current infection status of Arsenophonus sp. in honey bees. Following this, the diversity of Arsenophonus strains is ascertained through a multilocus sequence typing (MLST) scheme. Our further work will explore the biology and transmission modes of Arsenophonus sp., its effect on colony performance and suitability as a health marker. In partnership with the National Bee Unit, we hope to use the research to improve colony husbandry and devise strategies for the eradication or management of Arsenophonus sp. in honey bees.

S24/P8

The biology of host-shifts: a case of no return?

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Parasites, pathogens and indeed beneficial symbionts can all shift between host species. The frequency of host-shifts varies between different symbiont species. Additionally some symbionts are 'specialists', which move to only closely related host species, whereas others can make large jumps and are said to have a 'broad host range' (BHR). Plasmids that have a BHR are important for the spread of characteristics such as antibiotic resistance between distantly related bacteria. When a host shift occurs, the parasite/pathogen is placed in a very novel environment. We expect natural selection to then promote strains carrying mutations which allow them to persist in the host. In my PhD I seek to establish the extent of adaptation that occurs directly following a host shift event and whether this adaptation then makes it able to thrive in (and thus jump into) a related host. Finally, I wish to find out whether this adaptation makes it less able to prosper in (and jump back to) its ancestral host.

S24/P9

The gut microbiome of two Pachysoma spp. Macleay (Coleoptera: Scarabaeidae) feeding on different diets.

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Micro-organisms inhabiting the gut benefit from a protected nutrient rich environment while assisting the host with several roles, including nutritional and digestive functions. In this study we compare, for the first time, the bacterial and fungal gut communities of two Pachysoma (dung beetle) spp. feeding on different diets: P. endroedyi (polyphagous) and P. striatum (coprophagous). Whole gut microbial community structures were determined by 454 pyrosequencing of the bacterial 16S rRNA gene and the fungal ITS gene region. Both intra- and interspecific variation were noted for bacterial gut community structure. The number of bacterial phyla present within the gut ranged from 6-11 and 4-7 for P. endroedyi and P. striatum, respectively. The gut microbiomes were significantly different (ANOSIM R=1, $p<0.05$), with only 2.57% of the bacterial OTUs shared between the two Pachysoma species studied. Fungal communities could not be detected in the gut of P. endroedyi, while two fungal phyla could be detected within the gut of P. striatum. We are, as yet, unable to determine the extent to which host phylogeny or host diet is a determining factor in the structuring of Pachysoma species gut microbiomes, although the bacterial gut species diversity of the polyphagous feeder (P. endroedyi) is suggestive that host diet may be important in the structuring of the gut communities.

S25/P1

HIV P24 ANTIGEN AMONG HIV ANTIBODY SERONEGATIVE BLOOD DONORS IN OSOGBO OSUN STATE, SOUTH WESTERN NIGERIA

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Abstract
HIV transmission through transfusion of blood or its blood products is still a problem because of the inability of antibody based rapid methods to detect infection during window period. Transmission of HIV through infected blood and its products accounts for approximately 10% in African region.

Methods: This study analyzes true negativity of HIV infection in blood donors based on p24 core antigen detection. Four hundred and eighty blood donors initially negative for HIV antibody by rapid screening kit, Determine™ HIV-1/2 (Abbott Laboratory, IL, USA). The test results were confirmed with the Immuno Comb® II HIV 1 and 2 (Bispot kit PBS Organics and Israel 2005), All the 480 samples were further screened for the presence of HIV antibody and p24 HIV core antigen using ELISA kits (Genscreen TM ULTRA HIV Ag - Ab) following manufacturer's instructions. All donors initially tested negative for Hepatitis B virus (HBV), Hepatitis C virus (HCV).

Result: Out of the 480 blood donors in this study, 2(0.4%) were positive for the p24 HIV core antigen. The two positive donors for the p24 antigen had multiple sexual partners and recent sexually transmitted infections.

Conclusion: This study showed a low p24 antigenaemia (0.42%) among blood donors in Osogbo. Southwestern Nigeria.

Keywords: HIV P24 antigen, HIV Antibody, Seronegative Donors,

S25/P2

Centre for AIDS Reagents (CFAR)

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The Centre for AIDS Reagents (CFAR) is an internationally renowned HIV research repository based at the National Institute for Biological Standards and Control (NIBSC) in the UK. Since its inception in 1989 CFAR has supported HIV/AIDS research through the provision of thousands of high quality, bespoke research reagents to researchers worldwide.

CFAR houses over 6500 HIV/AIDS reagents, including extensive collections of virus strains, cell lines, antibodies, DNA clones, expression plasmids, recombinant proteins, peptides and anti-retroviral compounds. Which are stored and distributed in compliance with ISO9001 quality system. Most of these reagents have been donated by researchers for the benefit of the wider research community. In addition to provision of reagents CFAR also promotes the formation of partnerships between research groups and investigator driven networks in order to facilitate the development of vaccines and therapeutics.

CFAR provides a one-stop shop for scientists to disseminate reagents they have produced as well as obtain reagents for their own research. By managing the dissemination of materials CFAR assists researchers in overcoming the complicated regulations and logistic challenges involved in shipping infectious and other biological materials within a cold chain. Materials in the repository continue to support future research whilst acting as a legacy to the donor scientist.

Using the successful model of CFAR NIBSC is currently developing a new repository, through the EU FP7 EURIPRED project, for provision of reagents for poverty related diseases (Tuberculosis, Malaria, Hepatitis B/C).

S25/P3

Deep sequencing the whole viral genome of a heterologous challenge virus in Mauritian cynomolgus macaques identifies unique breakthrough variants after live attenuated SIV vaccination

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The SIVsmE660 viral stock represents a widely used heterologous challenge virus used in macaque models of HIV/AIDS for vaccine efficacy and pathogenesis studies. In Mauritian-derived cynomolgus macaques (Macaca fascicularis, MCM), SIVsmE660 displays unusual, highly reproducible kinetics replicating to high, persisting levels after intravenous inoculation. Yet vaccination with a live attenuated SIV is able to prevent SIVsmE660 superinfection in 75% of vaccinates. Where vaccine escape occurs, the breakthrough is similar to wild-type levels of vaccination. We have performed deep sequence analysis of SIVsmE660 breakthrough variants at 14 days post-inoculation in individuals vaccinated for either 21 days or 20 weeks. Sequencing was performed on overlapping amplicons spanning the entire SIVsmE660 genome using Illumina MiSeq and data was analysed using Geneious v7 or as per Genome Analysis Tool Kit best practice workflows. Vaccine escape variants identified 14 days post-challenge were compared with sequences recovered from a range of challenge inoculum doses in a separate in vivo titration study. Unique variants were identified at multiple locations not present in the inoculum challenge dose used as compared to the SIVsmE543 reference strain. The significance of different SNP frequencies identified in early and late vaccination regimes will be presented and discussed.

S25/P4

Modelling The Neuronal Impact of HIV Infection

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Antiretroviral therapy (ART) successfully suppresses viral load in HIV patients. However, it does not prevent many patients suffering chronic neurocognitive impairment (NCI), seriously impacting their daily life.

Using non-accelerated ART-free SIV models, we have demonstrated previously that neuro-invasion rapidly occurs and initiates a chronic progressive neuro-inflammation, despite the control of peripheral viremia. We have performed immuno-histochemical analyses to establish the effects of this inflammation on neuronal and synaptic integrity within regions associated with memory and motor function loss described in HIV-NCI.

First we studied brain collected from macaques immunised with SIV Gag vaccines before infection. Vaccination significantly blunted primary viremia, but did not reduce virus set point. Frontal lobe and cerebellar neuronal and synaptic staining patterns 5 months after infection were altered markedly amongst vaccine challenge controls compared with virus naïve individuals. Immuno-staining patterns from vaccinated macaques exhibited an intermediate pattern retaining some neuronal integrity.

In a follow-up study the kinetics of neuronal and synaptic changes in the hippocampus was examined following infection with attenuated SIV. Neuronal and synaptic changes occurred 3dpi, peaking 10dpi. Partial resolution was apparent by 21dpi but remained abnormal at 125dpi when plasma viremia had returned to undetectable levels.

Thus, we conclude that progressive neuronal and synaptic damage occurs rapidly in areas associated with memory and spatial awareness following SIV infection. Blunting the primary viremia through vaccination is beneficial but does not prevent it. ART alone may not prevent progressive neuropathology. Other complementary treatments are needed to protect against HIV-NCI.

S25/P5

Live attenuated SIV does not persist by occupying an immune privileged site in lymphoid tissue

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Model systems inform development of effective prophylactic vaccines against HIV/AIDS by defining correlates and mechanisms of protection. Live attenuated simian immunodeficiency virus (SIV) confers highly effective protection against pathogenic SIV challenge via multiple routes. The mechanism of protection remains elusive, however, the degree of protection is inversely correlated with the degree of attenuation and requires LAV persistence within secondary lymphoid tissues. In rhesus macaques (*Macaca mulatta*) it has been proposed LAV protection requires maintenance of high frequencies of effector CD8 T cells in secondary lymphoid tissues. Survival of LAV infected cells is associated with persistence in follicular T helper cells (TFH) located in immune privileged sites within follicular germinal centres (FGC).

We have examined the distribution of live attenuated SIV in lymphoid tissues in cynomolgus macaques (*Macaca fascicularis*) where robust and reproducible protection is detectable by 21 days after vaccination and fully established by 140 days. The distribution of LAV infected cells alters during the primary viremia. By day 10 LAV infected cells are regularly located within FGC. At day 21 and subsequently, LAV remain within FGC and additionally are frequently found within follicular marginal/mantle zones, a position where CD8 T cells and dendritic cells are co-located. Further work identifying the properties of LAV infected cells and the functional state of co-locating dendritic cells and CD8 T cells will be presented.

These data concur with cell depletion studies that do not support a central role of CD8 cells alone in the potent vaccine protection conferred by LAV in cynomolgus macaques.

S27/P1

Establishing a compendium of physiologically relevant RNA-seq data from *Paracoccus denitrificans* to inform N2O emission mitigation strategies

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Nitrous oxide (N2O) is a potent greenhouse gas, with a greater radiative potential than carbon dioxide, on a per-molecule basis. N2O emissions continue to rise by approximately 0.25% each year. Most of these emissions are from arable land, which contains a wide variety of denitrifying bacteria. These bacteria convert nitrogen in fertilisers to other forms including N2O. It is already well established that various environmental factors including pH, aeration and metal availability effects production of N2O at an enzymatic level. Despite this we lack detailed knowledge of the effects these factors can play at the transcriptional level. Recently, using microarrays, our laboratory demonstrated that copper availability effects transcription of the enzymes required for N2O production in the model denitrifier *Paracoccus denitrificans*. This current study expands on this work and attempts to obtain a global view of transcription in *Paracoccus denitrificans* using RNA-seq. RNA-seq allows high resolution mapping of the primary transcriptome, including identification of small RNAs. This approach has also allowed us to identify, for the first time, small RNAs in the *Paracoccus denitrificans* genome. 167 sRNAs have been identified which are either present in intergenic regions or located antisense to ORFs. Furthermore, many of these sRNAs are differentially expressed under different copper conditions. Future work will focus on validation of these sRNAs. Better understanding of the environmental factors which contribute to transcription of the N2O machinery will, in turn, help to inform strategies for mitigation of N2O emissions.

S27/P2

RNA-SEQ BASED TRANSCRIPTOME ANALYSIS OF THE INTERFERON HOST RESPONSE UPON VACCINIA VIRUS INFECTION.

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Evasion of interferon (IFN)-mediated antiviral immunity is critical for a successful virus infection. So poxviruses have evolved diverse molecular strategies to counteract IFN host response activity. In the case of VACV, one of these is to encode a soluble IFN-a/b binding protein (IFNBP) which located at cell surface binds type-I IFN molecules with high affinity, preventing their further interaction with the host cell receptor.

In the present work, we have dissected by RNA-Seq the viral modulation of the IFN-based host response at the transcriptional level. RNA from VACV-WR or a deletion mutant lacking the IFNBP infected L929 cells was extracted at 0, 4 or 9 hours post-infection in the absence or presence of IFN-alpha and/or recombinant purified IFNBP. Over 100M high quality reads per sample were obtained and mapped either to the VACV or *mus musculus* strain C57/BL6 reference genomes. Then, differential gene expression and GO pathway enrichment analysis were performed to reveal the mechanisms of action.

We could validate the experiment identifying the expected transcriptional changes in the transcriptome from IFN-treated cells. The addition of recombinant IFNBP to cells prior to IFN completely prevented these IFN-induced changes. The addition of recombinant IFNBP to cell cultures did not result in any significant activation of cellular pathways. Finally, to detect and analyze those changes in host gene expression after viral infection of cell cultures treated or not with IFN, differential gene expression and GO pathway enrichment analysis were performed and will be discussed.

S27/P3

Campylobacter jejuni flagellar biogenesis: the role of FlhF

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Amphitrichous *Campylobacter jejuni* lack the flagella biosynthesis master regulator (*flhDC*) found in well-studied model organisms such as *E. coli* and *Salmonella*. Like most polar flagellate bacteria, *C. jejun*i carry tandem flagellar genes Cj0064c:*flhF* and Cj0063c:*flhG* (a GTPase SRP-pathway subfamily protein, and a MinD homologue, respectively) shown to be involved in the regulation of flagellar biogenesis. Deletion of *flhF* by insertional mutagenesis caused an aflagellate and non-motile phenotype in the NCTC11168 strain. Pseudo-revertant isolates, obtained through repeated subculturing and selection of motility of the *ΔflhF* isolates, are motile and have a variety of flagellated phenotypes. Observation of biflagellated cells with non-polar flagellar placements confirms the role of FlhF as a polar landmark protein. Pseudo-revertant isolates are motile in semi-solid agar at mid-log phase and stationary phase; however no flagella are observed in liquid mid-log culture and low numbers of flagella are observed in liquid stationary culture. Despite this, many flagella were seen when cultures were taken from agar plates. Genome sequencing of 9 independently-derived pseudo-revertants revealed point mutations in flagella-associated genes. Eight of the nine pseudo-revertants had at least one non-synonymous point mutation in *fliF* and one had a similar mutation in *fliG*. These proteins encode the MS and C rings of the flagellar basal body. Trancriptomic analysis and *in silico* protein structure prediction suggests that structural and conformational properties of the MS and C ring-proteins play a key role in the completion of *C. jejuni* flagella through the induction of the σ54 regulon.

S27/P3

SUMOylation of Influenza A Virus Matrix Proteins

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SUMOylation is a posttranslational modification where SUMO a Small Ubiquitin-like Modifier is covalently bound to a lysine residue by a small set of known enzymes. SUMOylation regulates transcription, cell-cycle progression and DNA repair. Influenza A virus (IAV) replicates in the host cell nucleus and as SUMOs are predominantly located in the cell nucleus, multiple influenza virus proteins become SUMOylated. The IAV matrix protein (M1) is SUMOylated at K242. This SUMOylation is required for the interaction between M1 and RNP. A lack of M1 SUMOylation prevents the export of RNP. In addition to K242 there are 7 other conserved lysine's in the sequence of M1. Results from this project indicate that in addition to M1, additional proteins produced from M-segment splicing are SUMOylated, despite lacking the essential SUMOylated K242 reside of the M1 protein. These results have implications for protein function and suggest an effect of SUMOylation on viral protein-protein interactions during IAV assemlbly.

S27/P4

An RNA-seq based investigation of epigenetic regulation by the Type I restriction enzyme SpnD39III in *Streptococcus pneumoniae*.

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Streptococcus pneumoniae (the pneumococcus) is a major pathogen responsible for significant morbidity and mortality worldwide. We have demonstrated1 that a phase variable Type I restriction modification system (SpnD39III; *hsdRMS*), found ubiquitously in pneumococci, has multiple recombinable specificity alleles which direct adenine methylation of DNA at one of six distinct sites (SpnIIIA-F). Furthermore the pattern of these epigenetic modifications significantly alters virulence gene expression. The pneumococcus strain D39 was genetically modified to create six strains each "locked" with a single *hsdS* allele. Strains with a locked *hsdS* allele B have reduced capsule expression, show mainly transparent colonies and can asymptomatically colonise the nasopharynx; in contrast strains with a locked allele A have opaque colonies and preferentially cause systemic infection in mouse models.

We are using RNA-seq to investigate the regulatory mechanisms by which SpnIII-derived DNA methylation patterns influence gene expression in the pneumococcus. Previously we examined gene expression in D39 strains locked into a single hsdS allele, and we have now extended this to cell populations which are wildtype for the SpnD39III locus but which have significant enrichment for cells with the same *hsdS* allele active. We have also created single basepair frameshift (fs) mutations of the *hsdR* gene in SpnIIIA and SpnIIIB-locked strains of D39 and DP1004. Pneumococcal phage work has confirmed the *hsdR*-fs strains can still methylate at SpnIII sites, indicating an active HsdMS methylase; however they are unable to restrict unmodified phage confirming HsdR inactivation. Interestingly these *hsdR*-fs mutants show altered gene expression.

[1]Manso et al, 2014; doi:10.1038/ncomms6055

S27/P5

Functional Characterisation of TprA Regulators in *Streptococcus pneumoniae*.

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TprA is a transcriptional regulator of *Streptococcus pneumoniae*, and it is shown to be activated by the signaling peptide PhrA. However, the role of TprA/PhrA system in pneumococcal physiology and virulence is not known in detail. Hence, the objective of this study was to further the knowledge on the function of TprA/PhrA system. We show by reporter gene assays that TprA/PhrA system is active in the presence of galactose and mannose. TprA and PhrA mutant strains appear to be significantly attenuated in growth on glucose, galactose, maltose, and N-acetylglucosamine compare with the wild type D39 strain. In addition, we also demonstrated that mutation of *tprA* abrogates pneumococcal virulence in a mouse model of pneumococcal pneumonia that develops after intranasal infection as well as in septicemia model. Analysis of TprA mutant indicated that the mutant had decreased production of important virulence factors such as neuraminidase and hyaluronidase compared to wild type D39 strain. Our results show that TprA is crucial for pneumococcal host-derived sugar metabolism, and virulence.

S27/P6

Comparative multi-condition inter-strain transcriptomics identifies unexpected differences between global and African sequence types of *Salmonella* Typhimurium

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Salmonella Typhimurium infects a wide range of animal hosts, and generally causes a self-limiting gastroenteritis in humans. However, a variant of this serovar, ST313, is causing an emerging invasive *Salmonella* disease in sub-Saharan Africa that targets susceptible HIV+, malarial or malnourished individuals. A genomic comparison between an ST313 isolate, D23580, and the well-characterized gastroenteritis isolate 4/74 (ST19) showed that the two strains share 96% of coding genes. Genetic differences include 1000 SNPs, two D23580-specific prophages and the presence of pseudogenes.

To investigate the hypothesis that altered gene expression patterns reveal distinct virulence mechanisms between ST19 and ST313, RNA-seq-based transcriptomic data were obtained for strains 4/74[1,2] and D23580, grown under seventeen infection-relevant *in vitro* conditions and during infection of macrophages. Key transcriptomic data were validated with a proteomic approach.

Comparative transcriptomics of the two bacterial strains showed that between 1 to 6% of all ST19 and ST313 genes were differentially-expressed in individual stress conditions. Early stationary phase (ESP) is a condition that induces the SPI1-encoded invasion system, and 2% of genes showed differential expression. Proteomics confirmed that 16% of the differentially-expressed genes in the ESP condition also showed altered expression at the protein level.

We are investigating whether the differences observed in gene expression of virulence-associated genes under specific environmental conditions reflect the distinct pathogenic mechanisms of these two *Salmonella* strains.

References:
[1]Kröger *et al* (2013) *Cell Host Microbe* 14:683-95.
[2]Srikumar *et al* (2015) *PLoS Pathog* 11:e1005262.

S28/P1

High-specificity genome editing of herpes simplex viruses using the CRISPR/Cas9 system

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The generation of large DNA viruses expressing mutant versions of endogenous or foreign genes have proven a useful tool to understand viral pathogenesis, characterizing viral proteins that interact and modulate the host immune system. Herpes simplex viruses (HSVs) are prevalent human pathogens of clinical relevance, frequently used as a model to study viral pathogenesis and modulation of the host immune response. Traditionally, homologous recombination was used as the main method for the construction of viral mutants, with low efficient rates. Bacterial artificial chromosomes (BACs) have also been used to generate recombinant viruses, but residual BAC sequences or the loss of viral endogenous regions may cause viral attenuation.

The CRISPR/Cas9 genome-editing tool has been proposed as a powerful alternative to generate efficiently recombinant viruses. We have selected the US4 gene from HSV-1 and HSV-2, encoding glycoprotein G (gG), as the target locus. gG binds chemokines and enhances their activity, and represents an excellent candidate to study viral immune modulation.

Knock-in viruses, in which the US4 gene was replaced by an eGFP cassette as a selection marker, were generated using the CRISPR/Cas9 system through the transfection-infection method. We have satisfactorily replaced the US4 gene by eGFP, selecting fluorescent plaque-purified recombinant viruses. We have sequenced by next generation sequencing (NGS) the recombinant viral genomes and confirmed the correct insertion of eGFP and the absence of alterations elsewhere in the genome. These results support the CRISPR/Cas9 genome editing system as a useful approach to generate HSV-1 and HSV-2 recombinants.

S28/P2

Natural Distribution of Group II Introns and Application as Genetic Tool in Actinobacteria

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Actinobacteria include many specialised metabolite producers and are a likely source of novel bioactive substances. Due to the advancements in next generation sequencing techniques, increasing numbers of genomes have been sequenced from this bacterial group. Despite this, there is still a lack of genetic tools for many species.

Group II Introns are a class of catalytic RNAs, capable of self-splicing and retrohoming to a specific target site. These retro-elements usually contain a multifunctional intron encoded protein (IEP) containing maturase, DNA binding, reverse transcriptase and endonuclease domains.

Due to their specificity and retrohoming efficiency, Group II introns are of interest for use as genetic tools. So far they have been successfully used for gene disruptions and deletions in both Gram- positive and Gram- negative bacteria and a commercial system has been developed.

The aim of this study was to find out if these elements can be used in *Streptomyces* and other high GC content actinobacteria. We constructed a synthetic Group II intron for actinobacteria by codon-optimising of IEP and a suitable resistance gene. As a proof of principle the undecylprodigiosin regulator *redD* was used as target gene in the model species *S. coelicolor*.

Furthermore we analysed the natural distribution of potential group II introns within actinobacteria by searching for the IEP, after alignment, phylogenetic analyses were carried out. This revealed great sequence variety, as well as presence of lateral gene transfer due to non-species like topology suggesting, that at least some of the detected IEPs are part of functional introns.

S29/P1

Analyzing Epitope of Dengue Virus Serotype 1 and 2 as the Basis for Vaccine Development: A Descriptive Study of Partial Envelope (Domain III)

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Introduction:

Epitope study of dengue virus (DENV) is required as a basis to develop a suitable structure for vaccine development. Domain III (partial of the envelope) is the most important properties of Envelope protein, which plays role as mediating virus entry and in inducing long-lasting protective immunity against dengue virus infection. This partial of the envelope that lay at the surface of the virus, is believed to be a genotype-specific and serotype-specific complex. This study aims to obtain data about domain III epitope of DENV-1 and DENV-2 as the basis of the vaccine development.

Methods:

We took the sample from GenBank (http://www.ncbi.nlm.nih.gov/genbank) and Laboratory of Microbiology, Faculty of Medicine Universitas Indonesia. There were 30 sequences of DENV-1 and 42 sequences of DENV-2, selected to represent each genotype based on sequences from Goncalvez et al. and Wu et al. as references for determining genotypes. Genetyx 5.1 was used for processing the amino acid homology of the sequences. Then we used study from Li et al to determine the location of the epitope of DENV domain III.

Results:

Results showed that no mutation was occurred on amino acid of DENV-1 Domain III epitope at ^{309}LEKEVAETQHGTV^{320} and also, with DENV-2 Domain III epitope at ^{309}KEIAETTQHGTI^{320}.

Conclusion:

From this study, we can conclude that the epitopes from both serotypes are conserve. Thus, the domain III can be an important basis for the development of multivalent dengue vaccine.

S29/P2

The effect of temperature on the replication kinetics of Rift Valley fever virus in vertebrate and invertebrate cell lines

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Rift Valley fever virus (RVFV) is a pathogen of both veterinary and public health importance causing widespread abortions and mortality in ruminants. In humans it causes an acute febrile illness, with severe complications in 1% of cases. RVFV is endemic throughout Africa and its introduction to the Arabian Peninsula in 2000 demonstrates the ability of this virus to cross geographic barriers and establish in previously non-endemic regions raising concerns for its introduction into Europe. The virus is maintained in a transmission cycle between its mosquito vector and mammalian hosts. Temperature is a fundamental factor when considering the transmission potential of temperate mosquito species encountered in Europe. Genomic mutations within the virus could lead to adaptation promoting survival in temperate regions.

We studied the effects of temperature on RVFV growth kinetics at 12oC and 20oC in mosquito cells and 28oC and 37oC in mammalian and mosquito cell lines. Infectious viral particles were quantified by plaque assay and genome equivalent copies by real-time RT-PCR. RVFV replicated at all temperatures however reduced temperatures correlated with an increased latent period and lower viral titres. To investigate mutations associated with growth at different temperatures, we developed an NGS strategy utilising four RT-PCRs to amplify the tripartite genome. A single amplicon for the complete S (1690bp) and M segments (3885bp) and two overlapping amplicons for the L segment (3449bp and 2923bp). This technique was applied to sequence strains of RVFV and will be utilised to monitor sequence variation associated with reduced replication observed at lower temperatures.

S29/P3

Structure based drug design for Nairovirus nucleocapsid proteins

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Crimean-Congo haemorrhagic fever virus (CCHFV) is the causative agent of Crimean-Congo haemorrhagic fever. With a mortality rate reaching 70% and a broad geographical distribution, CCHFV is classified as a BSL-4 pathogen due to its high lethality and lack of effective treatments. CCHFV is a segmented negative stranded RNA virus, with its genome separated into three RNA strands, named small (S), medium (M) and large (L); S encodes the nucleoprotein (N), M encodes the glycoproteins and L encodes the RNA-dependent RNA

polymerase. N encapsidates the RNA segments, which is critical for production of infectious virus particles, and thus N represents a promising therapeutic target. CCHFV shares its serogroup with Hazara virus (HAZV), which does not cause disease in humans and is consequently a BSL-2 pathogen. The N proteins of HAZV and CCHFV exhibit high structural homology, validating the use of HAZV as a CCHFV model. Structural data presented here indicates a defined pocket is responsible for binding RNA, ideal for structure based drug design (SBDD). RNA binding was analysed using HAZV and CCHFV N, the results demonstrate a potential high-throughput-screening assay to assess the inhibitory affects of compounds highlighted by initial rounds of SBDD.

S29/P4

Evaluation of a lentiviral based fusion assay as a novel neutralisation assay for Ebola virus

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Non-replicative retroviruses carrying the Envelope protein of a heterologous virus have been extensively used for the study of virus-host interactions, for gene therapy, and more recently for investigating neutralising antibodies. The use of these pseudotyped viruses expressing the envelope protein of a highly pathogenic wild type virus allows neutralisation assays to take place at a lower biological containment level (BSL2) than that required for the wild type virus (BSL3/4). For instance, candidate materials for the establishment of WHO Reference reagent for Ebola virus serology have been evaluated in-house prior to their inclusion in an International collaborative study using a neutralisation assay based on a lentiviral vector expressing the Ebola virus glycoprotein GP. Following the results obtained from the collaborating laboratories using different neutralisation assays, we are developing an alternative procedure for Ebola virus serology to increase sensitivity and inter-laboratory consistency. The assay measures the entry of an Ebola virus GP-pseudotyped lentivirus by luciferase activity in a reporter cell line. Therefore, the assay focuses only on the activity of neutralising antibodies which block receptor mediated-entry, independent from post-fusion events which may affect infectivity and rely on the expression of a reporter gene driven by a promoter in the vector. The serology panel investigated for the establishment of the WHO Reference reagent for anti-Ebola virus antibodies will be used to evaluate sensitivity and reproducibility of the fusion-based neutralisation assay.