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Abstracts

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This Abstracts Book has all the Session Abstracts in the first section and all the Poster Abstracts in the second section. The Session abstracts are ordered by session then by the day and time of presentation. The Poster abstracts are ordered by session/category then by the poster number.

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Session abstracts Session code – Day code – Time
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Poster abstracts Session or Category code / Number
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Prize and Hot Topic Lectures

SU00Mo1210 – Fred Griffith Prize Lecture

Breaking the mould in medical mycology

Neil A.R. Gow

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When Fred Griffith was carrying out his pioneering work on the epidemiology and pathology of bacterial pneumonia in the 1930's medical mycology was almost invisible in comparison to the well-established fields of medical bacteriology, virology and parasitology. However, fungal infections now account collectively for more annual deaths than malaria or tuberculosis and they represent a significant worldwide challenge and health burden. The sharp rise in the prevalence of life threatening fungal infections tracks the emergence of large numbers of AIDS infections and other immune compromised individuals, who are often particularly vulnerable to life-threatening fungal infections. Recent efforts have begun to address these clinical needs, and have established medical mycology and fungal immunology as fertile areas of basic and applied research that have contributed broadly to our appreciation of host-microbe interactions and microbial pathogenesis. Using some vignettes from recent research highlights and contributions from my own group's work, this lecture will attempt to demonstrate how efforts to understand and undermine fungal disease processes are underlining the credentials of medically important fungi such as *Candida*, *Aspergillus* and *Cryptococcus* species as pathogens to rival the best known bacterial, viral and parasitic pathogens of the modern world.

SU00Tu1210 – Hot Topic Lecture

Fungi challenge food security and plant ecosystem health: disease, die-back and distribution

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Fungal diseases have been increasing in severity and scale since the mid 20th Century and now pose a serious challenge to global food security and ecosystem health (Gurr *et al*., 2011, *Fungal Biology Reviews* **25** 181-188). Indeed, we have demonstrated recently that the threat to plants of fungal infection has now reached a level that outstrips that posed by bacterial and viral diseases combined (Fisher *et al*., 2012 *Nature* **484** 185-194).

This presentation will highlight some of the more notable persistent fungal and oomycete plant diseases of our times. It will draw attention to the emergence of new pathotypes affecting crop yields and to fungi and oomycetes decimating our natural and managed landscapes. I shall review some of our recent work looking at the movement of pests and pathogens polewards in a warming world (Bebber, Ramatowski and Gurr *under review*) and at the global distributions of crop pests and pathogens (Bebber *et al, under review*).

I shall conclude with some thoughts on the recent emergence of the ash die-back fungus and comment on the findings of the Nomex consortium with regards to the biology of infection and the search for host disease resistance.

SU04Tu1105 – Outreach Prize Lecture

A rough guide to outreach

Helen Louise Brown

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Participation in outreach has become an important aspect of research. Outreach activities allow the public to see how research works, and increase accountability and transparency. From the researcher's point of view, benefits to outreach participation are: greater public understanding of research, improving communication skills, and speaking to different audiences.

The British media are criticised for their narrow focus and sensationalism in microbiological reporting. Despite this, their reporting has led to increased public interest in microbiology. This interest means that there is a great potential for public engagement, with a broad and varied audience attending events.

Grants commonly specify that public engagement must be carried out alongside scientific research. To fulfil these requirements it is important to encourage and support scientists taking part in outreach. In order to do this we need to overcome many of the traditional barriers to engagement.

In this talk I will discuss my motivation for participating in outreach and will discuss some of the events I have been involved with. I will also highlight how local and national networks as well as societies are able to provide support and resources to researchers wishing to take part in a wide variety of outreach events.

SU04Tu1120 – Outreach Prize Lecture

The Good, the Bad and the Algae: a public engagement event

James Redfern

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Science festivals and similar events provide excellent opportunities for the public to engage with 'hands-on' microbiology. However, in addition to safety concerns, many microorganisms can be difficult to visualise without appropriate equipment. Algae are safe to handle, relatively large and sufficiently different in appearance to enable viewing under a microscope with little technical ability. As part of the 2011 National Science and Engineering Week a public engagement event, 'The Good, The Bad and The Algae' was delivered in MMU's microbiology laboratory. After an introduction, participants identified algae in samples using a key which had been previously developed for use in schools. The feedback (free-text and verbal) was overwhelmingly positive. The activity was redeveloped from a 'workshop' to a 'drop-in' event, and presented at the national Big Bang Science Fair in partnership with the Society for General Microbiology. Additions included LCD screen microscopes, 3D images and a modelling to scale activity. Engagement and learning were assessed numerically, through dialogue, and through free-text feedback: information indicated a positive experience. Over 2,200 people interacted with the activity over three days.

Sessions

SU01
Microbial modulation of cellular pathways

SU01Mo0900

Bacterial protein toxins targeting actin and regulatory GTPases

Klaus Aktories

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The cytoskeleton is essential for cell morphology and motility. It is crucially for epithelial barrier functions and immune cell signaling. The cytoskeleton (especially the actin cytoskeleton) is a main target of bacterial protein toxins. Many toxins manipulate the actin cytoskeleton by targeting GTP-binding proteins of the Rho family, which are master regulators of the actin cytoskeleton. The toxins cause ADP-ribosylation, glucosylation, adenylylation, deamidation and proteolysis of Rho proteins, thereby activating or inactivating the switch proteins. Other bacterial toxins alter Rho proteins by hijacking functions of endogenous regulators of Rho protein activity. Actin is also directly modified by bacterial protein toxins, which cause ADP-ribosylation of actin at arginine177 or threonine148, thereby actin polymerisation is inhibited or facilitated. The presentation focuses on modification of actin and of Rho proteins induced by bacterial protein toxins produced by Clostridia and Photorhabdus.

SU01Mo0930

A *Burkholderia pseudomallei* toxin deamidates eIF4A

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Burkholderia pseudomallei is the causative agent of melioidosis, a disease of human and animals which is increasingly reported in many tropical and sub-tropical countries. In humans the disease is often fatal despite aggressive antibiotic treatment. Over the past 50 years there has been interest in virulence mechanisms of *B. pseudomallei* and during the 1950s and 1960s several workers reported that the bacterium produced an exotoxin. However, the identity of this toxin was not determined. We have identified a protein (BPSL1549) which is toxic to mice and to cultured cells. This protein shows structural homology with catalytic domain of *E. coli* cytotoxic necrotising factor 1 (CNF1) including conservation of active site catalytic residues. The mutation of one of these residues, cysteine 94, markedly reduced the toxic activity of BPSL1549. The cellular target for *B. pseudomallei* BPSL1549 was different than that for *E. coli* CNF1. Whereas CNF1 deamidates Rho GTPases BPSL1549 deamidates eIF4A, blocking translation. The role of BPSL1549 in virulence is still being investigated, this protein may be a target for vaccines and antimicrobials.

SU01Mo1000

Action of biopesticides at molecular level

Christos Gatsogiannis[1,*], Alexander E Lang[2,*], Dominic Meusch[1], Vanda Pfaumann[2], Oliver Hofnagel[1], Roland Benz[3], Klaus Aktories[2], STEFAN RAUNSER[1]

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Photorhabdus luminescens is an insect pathogenic bacterium that is symbiotic with entomopathogenic nematodes[1]. Upon invasion of insect larvae, *P. luminescens* is released from the nematodes and kills the insect through the action of large tripartite ABC-type toxin complexes (Tcs)[2]. Tcs are typically composed of TcA-, TcB- and TcC proteins. Functioning as ADP-ribosyltransferases, TcC proteins were identified as the actual functional components that induce actin-clustering and cell death[3]. However, little is known about the translocation of TcC into the cell by the TcA and TcB components. Here, we show that TcA (TcdA1) forms a transmembrane pore and report its structure in the prepore and pore state determined by cryo-electron microscopy[4]. We found that the TcdA1 prepore assembles as a pentamer forming a α-helical vuvuzela-shaped channel less than 1.5 nm in diameter surrounded by a large outer shell. Comparisons with structures of the TcdA1 pore inserted into a membrane and in complex with TcdB2 and TccC3 reveal large conformational changes during membrane insertion suggesting a novel syringe-like mechanism of protein translocation. Our results demonstrate how ABC-type toxin complexes bridge a membrane to insert their deadly components into the cytoplasm of the host cell. Our proposed mechanism is paradigmatic for the whole ABC-type toxin family. It is an important step towards the understanding of the host-pathogen interaction and the complex life cycle of *Photorhabdus luminescens* and other pathogens, including human pathogenic bacteria, and serves as a strong foundation for the development of biopesticides.

1) Joyce, S. A., Watson, R. J. & Clarke, D. J. *Curr Opin Microbiol* **9**, 127–132 (2006); 2) ffrench-Constant, R. H. & Bowen, D. J. *Cell. Mol. Life Sci.* **57**, 828–833 (2000); 3) Lang, A. E. *et al.. Science* **327**, 1139–1142 (2010); 4) Gatsogiannis, C. *et al.. Nature* **495**(7442): 520-23 (2013).

SU01Mo1100

The ins and outs of pertussis toxin

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Pertussis toxin is a unique toxin, solely produced by the Gram negative microorganism *Bordetella pertussis*, the etiological agent of whooping cough. It is also the most complex bacterial exported protein known so far, composed of five different subunits assembled into an A-B structure. The toxin is secreted by *B. pertussis* via a type IV secretion system, encoded by the *ptl* genes located just downstream of the toxin structural genes. Via the B oligomer, composed of subunits S2 to S5, the toxin binds to a variety of cellular receptors, which leads to intracellular trafficking of the toxin via receptor-mediated endocytosis and retrograde transport. By a non yet fully elucidated mechanism, S1 then translocates through the membrane. Upon translocation, it catalyses the transfer of the ADP-ribosyl moiety of NAD^+ onto to alpha subunit of Gi/Go proteins, which locks them in the inactive state. Depending on the target cells, this results in a variety of biological activities, such as islet activation, lymphocytosis, histamine sensitisation and others. Pertussis toxin is a major virulence factor of *B. pertussis* and interferes both with innate and adaptive immune responses to this organism. In addition, it is a protective antigen and is present in all current pertussis vaccines.

SU01Mo1130

Pasteurella multocida toxin and its potential link with cancer

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The concept that bacterial infection could cause cancer has only recently become accepted because of the strong epidemiological and molecular evidence for the role of *Helicobacter pylori* in gastric cancer. *Salmonella typhi* is the only other infection linked to human cancers, with *S. typhi* carriers showing a high incidence of hepatobiliary carcinoma. Information on other potential bacterial carcinogens is very limited. A different approach is to assess bacteria for potentially pro-carcinogenic properties. The *Pasteurella multocida* toxin (PMT) has many such properties. It is a highly potent mitogen that blocks apoptosis. PMT modifies and activates members of three of the four families of heterotrimeric G-proteins, all of which have potential roles in carcinogenesis. Indeed the residue altered by PMT is mutated in some cancers. Many downstream signalling components are known proto-oncogenes and have been shown to be activated by PMT. These include the Rho GTPase, focal adhesion kinase, cyclooxygenase-2, beta-catenin and calcium signalling. PMT action potentially influences many of the acquired Hanahan/Weinberg capabilities necessary for oncogenic transformation. As PMT can activate so many pathways linked to cancer, it is likely to have carcinogenic potential, and serves as an important novel paradigm as a bacterial pathogen linking deregulated cell signalling to cancer.

SU01Mo1400

Offered paper **Analysis of the accessory secretion system and novel secreted proteins in *Streptococcus***

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The bulk of protein secretion in bacteria is via the general secretion (Sec) pathway. Proteins are led to the Sec pathway by an N-terminal signal peptide, where they undergo processing and folding within the cell wall environment. Gram-positive bacteria have been shown to possess more than one Sec system. The alternate system, known as the accessory secretion system, may secrete a specific set of extracellular proteins, some of which lack a leader peptide. The core component of this system is SecA2 protein, a homologue of SecA from the canonical Sec system. This study aimed to determine the specificity of accessory SecA2 system in *Streptococcus gordonii* and *S. pneumoniae* for secretion of proteins with a conventional or non-conventional signal peptide. Knockout *secA2* mutants were generated to investigate the role of SecA2 in protein secretion, adhesion and biofilm formation. Adherence assays and mass spectrometry analysis confirmed that known SecA2-dependent surface proteins Hsa (*S. gordonii*) and PsrP (*S. pneumoniae*) were not secreted when *secA2* was inactivated. Haemolytic assays suggested that SecA2 may be involved in release of pneumolysin, a *S. pneumoniae* toxin lacking a signal peptide. Additionally, SecA2 was implicated in biofilm formation, a phenotype often associated with bacterial colonisation and pathogenic capabilities.

SU01Mo1415

Offered paper **Characterisation of the Bsa Type Three system secretome of *Burkholderia pseudomallei* using hyper-secreting mutants**

CHARLES VANDER BROEK, Mark Stevens, Joanne Stevens

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Many Gram-negative bacteria utilise Type III secretion systems (T3SS's) to deliver effector proteins into target host cells where they hijack cellular processes for their own benefit. The melioidosis pathogen *B. pseudomallei* possesses three T3SS's, of which the so-called Bsa system has been shown to play a role in invasion, escape from the endocytic compartment and virulence in a murine models of infection. Yet, few proteins have been proven to be secreted by the Bsa apparatus. We have determined the effector secretome of the Bsa T3SS using iTRAQ, a gel free quantitative proteomics technique. A T3SS needle-tip mutant (*bipD*) and a translocator: effector switch mutant (*bsaP*), the latter of which mimics the effects of targeted deletion of *sepL* in pathogenic *E. coli*, have been used as they hyper-secrete the known effector protein BopE. Secreted protein profiles from these hyper-secreting mutants have been compared to the isogenic parent strain and a T3SS structural mutant (*bsaZ*) to identify probable Bsa effector proteins. From this analysis we provide a comprehensive general secretome for *B. pseudomallei* including phage components, peptidases, flagella components, type I fimbriae and secreted lipoproteins. Furthermore we have identified 53 putative Bsa effector proteins, of which 44 are completely novel.

SU01Mo1430

Offered paper ***Helicobacter pylori* down-regulates expression of human β-defensin 1 in the gastric mucosa**

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Helicobacter pylori establishes a chronic infection in the human gastric mucosa, which may lead to peptic ulcer disease or gastric adenocarcinoma. The human beta-defensins (hβDs) are antimicrobial peptides; hβD1 is constitutively expressed in the human stomach. We hypothesised that down-regulation of gastric hβD1 expression may contribute to *H. pylori* persistence. *H. pylori* density, inflammation level and hβD1 expression were measured in gastric biopsies from 31 *H. pylori*-infected and 23 uninfected patients. In vitro co-cultures with human gastric epithelial cell lines examined the role of *H. pylori* virulence factors and the cell signalling pathways involved.

Significantly lower hβD1 mRNA levels ($p=0.005$) and protein concentrations ($p=0.001$) were present in gastric biopsies from infected patients. Biopsies from those with high bacterial colonisation densities and more severe inflammation expressed the least hβD1. *H. pylori* infection of human gastric epithelial cell lines in vitro also considerably down-regulated hβD1. The use of wild-type strains and isogenic mutants demonstrated that the *cag* pathogenicity island (*cag*PAI) is necessary for hβD1 down-regulation. Treatment with chemical inhibitors or siRNA duplexes revealed that the mechanism of hβD1 suppression involved *cag*PAI-induced NF-κB signalling.

H. pylori down-regulation of hβD1 expression via NF-κB signalling may promote bacterial survival and persistence in the gastric niche.

SU01Mo1445

Offered paper **Novel mechanism of innate immune response suppression by the Buruli ulcer toxin mycolactone**

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Buruli Ulcer, caused by *Mycobacterium ulcerans,* is the third most common mycobacterial disease in the world. Infection is characterised by necrotising ulceration combined with a profound inhibition of both innate and acquired immune responses. This is attributable to the production of the cytotoxic polyketide macrolide, mycolactone. Our previous studies in monocytes showed that sub-toxic levels of

mycolactone inhibit the LPS-dependent production of several key proinflammatory mediators including TNFα, IL6 and COX2 via a post-transcriptional mechanism. To investigate the possibility that mycolactone selectively suppresses translation, we compared polysome profiles between cells treated or not with mycolactone prior to LPS stimulation of the cells. Unexpectedly, northern blotting for TNFα, IL6 and COX-2 mRNA revealed that, despite a complete block in protein production, all three transcripts were primarily located in polysomal fractions from mycolactone exposed cells. This association was sensitive to translational disruptors (homoharringtonine and puromycin), suggesting that the mRNA is being actively translated. Furthermore, in digitonin-permeabilised cells all three mRNAs colocalise with the membrane fraction, suggesting nascent chain-directed endoplasmic reticulum targeting. We now present evidence that mycolactone targets secreted protein production co-translationally.

SU01Mo1500

Offered paper **Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 attaching/effacing (A/E) lesion formation and regulation of innate immune gene expression in human colonic epithelial cells**

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Enterohaemorrhagic *Escherichia coli* (EHEC) causes severe pathology, including haemorrhagic colitis (HC), in humans. Infection is associated with subversion of cellular pathways leading to formation of attaching/effacing (A/E) lesions, characterised by intimate adherence, microvilli effacement and actin polymerisation. Infection studies using human cell lines and mucosal biopsies have detected A/E lesion formation in intestinal epithelial cells (IECs) from small intestine but not the colon, the site of HC. EHEC has also been shown to affect interleukin-8 (IL-8) expression in colonic T84 cells but no other innate immune genes have so far been investigated. The aim of this project was to investigate A/E lesion formation and innate immune gene expression in human colonic IECs following infection with EHEC. Infection of polarised T84 cells revealed signs of microvilli effacement, but not actin polymerisation, around adherent EHEC. *In vitro* organ culture (IVOC) of biopsies yielded the first evidence of EHEC colonisation of human colonic tissue, which was also associated with microvilli effacement. EHEC infection resulted in increased mRNA expression of both IL-8 and the antimicrobial peptide, human β-defensin (hBD)-2, in T84 cells. Put together, this study shows that EHEC induces signs of A/E lesion formation and hBD2 expression in colonic IECs.

SU01Mo1545

Offered paper ***Staphylococcus aureus* superantigen SEIX inhibits neutrophil function**

STEPHEN NUTBEAM-TUFFS[1], Jovanka Bestebroer[2], Ivan Morrison[1], Jos Van Strijp[2], Ross Fitzgerald[1]
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Bacterial superantigens (SAgs) are a family of toxins which induce non-specific T-cell proliferation. It is likely that SAgs made by *Staphylococcus aureus* contribute to persistence by host immune avoidance but some SAgs are associated with serious life-threatening toxinoses such as toxic shock syndrome. Staphylococcal enterotoxin-like toxin X (SEIX) is unique among staphylococcal SAgs as it is encoded in the genome of >95% of *S. aureus* strains from both human and animal infections. SEIX is related to another group of immune modulatory proteins with structural homology to SAgs known as Staphylococcal superantigen-like proteins (SSLs). In the current study western blot analysis of deletion mutants of different S. aureus gene regulators indicated that SEIX expression is controlled by *saeRS*, a two component regulator which is linked to the bacterial response to phagocytic signals. Considering the co-regulation of SEIX with known mediators of innate immune avoidance, we investigated a potential role for SEIX in innate immune cell interactions. We discovered that SEIX strongly binds to human neutrophils and blocks phagocytosis. This establishes a function for SEIX in *S. aureus* pathogenesis independent of superantigenicity. Current work is aimed at identifying the host receptors involved and exploring the importance of this interaction in staphylococcal infection.

SU01Mo1600

Offered paper **Modulation of epithelial cell cycle and migration by meningococcal outer membrane protein PorA**

MATTHEW J VASSEY, Lee Wheldon, Akhmed Aslam, Jafar Mahdavi, Neil Oldfield, Dlawer Ala'Aldeen, Karl Wooldridge
University of Nottingham, Nottingham, UK

The cell surface laminin receptor (LamR) receptor plays an important role in cell adhesion to the basement membrane, signal transduction and cell movement. We recently showed that meningococci bind LamR and identified outer membrane porin protein, PorA, as one of two meningococcal LamR-binding ligands. Further studies utilising recombinant PorA sub-fragments and synthetic peptides localised the LamR-binding domain to the hyper-variable, surface-exposed fourth extracellular loop of this protein.

A linear wound healing assay was used to investigate the *in vitro* effect of PorA loop 4 on human epithelial cells. Wound closure was significantly reduced in cells treated with synthetic PorA loop 4. In contrast, recombinant PorA or synthetic loop 1 peptide had no effect on wounds closure.

Treatment with PorA loop 4 – but not loop 1, or scrambled Loop 4 peptide – resulted in a significant reduction in the number of cells synthesising DNA. Specifically, Loop 4 blocked the cell cycle at the G_2/M checkpoint while a scrambled Loop 4 peptide had no effect on cellular proliferation.

PorA loop 4-mediated effects the cell cycle and cellular migration, this is likely mediated through LamR and may play an important role in the interaction between *N. meningitidis* and human cells *in vivo*.

SU01Mo1615

Offered paper **Exploitation of host cell lipids by *Legionella pneumophila***

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Legionella pneumophila is an important human pathogen which causes a potentially fatal pneumonia, named Legionnaires' disease. Upon inhalation, *L. pneumophila* infects alveolar macrophages, in which it resides and replicates in a specialised vacuole, the so called *Legionella*-containing vacuole (LCV). Virulence and creation of the LCV essentially depend on a sophisticated type IVB secretion system which translocates more than 300 effector proteins into host cells. The function of most of these effectors is unknown, however the exploitation and manipulation of host cell lipids by effectors is an emerging theme. Here, we show that the conserved effector LtpL, which is predicted to belong to the phospholipase D (PLD) family, is

important for efficient replication of *L. pneumophila* strain 130b in A/J mice. Using TLC assays we confirm that LtpL has lipase activity and hydrolyses phosphatidyl glycerol (PG). Although only translocated into host cells at low levels, LtpL was detected localising to small vesicles at late stages of infection. Similarly, LtpL localised to vesicular structures, but also to the plasma membrane upon ectopic expression. Active LtpL but not a catalytically inactive mutant caused disruption of the Golgi apparatus, suggesting a strong effect of LtpL on host lipid signalling.

SU01Mo1630

Offered paper **Bacterial flagella penetrate into host cells and interact with cytoskeletal components**

ELIZA WOLFSON, Johanna Elvidge, Amin Tahoun, Trudi Gillespie, Edith Paxton, Fiona Lane, Darren Shaw, Andrew Gill, Jo Stevens, David Gally, Arvind Mahajan

University of Edinburgh, Edinburgh, UK

Bacterial flagella contribute to host pathogenesis in multiple ways, including chemotaxis, adherence and immuno-modulation. In this study, flagella-based adherence to epithelial cells by pathogenic *Escherichia coli* and *Salmonella* Typhimurium was investigated. Bacteria-associated flagella were observed to penetrate into and out of epithelial cells. Cellular ligand candidates were identified and were exclusively actin or actin-binding proteins. *E. coli* H6 and H7 flagella and *S.* Typhimurium phase-1 and phase-2 flagella were demonstrated to have differential interactions with cofilin-1, β/γ-actin and galectin-4, by far-Western blots and ELISAs. In vitro actin polymerisation assays revealed that S. Typhimurium phase-2 flagella enhanced actin polymerisation rates to a greater extent than the other flagella tested. There was also evidence of a filament-stabilising effect of cofilin-1 on H7 flagella. The contribution of H6 and H7 flagella to host colonisation has been highlighted in previous studies (Giron *et al.*. (2002) *Mol. Microbiol.* 44(2):361-379; Mahajan *et al.*. (2009) *Cell. Microbiol.* 11(1):121-137). Here we demonstrate that S. Typhimurium phase-1 and phase-2 flagellin mutants show reduced binding and invasion of epithelial cells. Taken together, sub-cellular penetration of flagella and differential interactions with cytoskeletal components contribute to initial bacterial binding and may facilitate subsequent stages of the infection process of these enteropathogens.

SU01Mo1645

Offered paper **A unique homologue of eukaryotic ubiquitin produced by *Bacteroides fragilis*, a commensal bacterium of the human gastro-intestinal tract**

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Bacteroides fragilis is an anaerobic, Gram-negative commensal bacterium of the human gastro-intestinal tract. *B. fragilis* encodes and expresses a protein (BfUbb), which has 63% identity to human ubiquitin. Genome sequencing and PCR shows that some, but not all, *B. fragilis* isolates contain the *ubb* gene. BfUbb has a predicted signal sequence and can be detected in whole cell extracts and supernatants containing outer membrane vesicles. These vesicles may act as a delivery system for ubiquitin, allowing *B. fragilis* to interact with the host. At present the mechanism of BfUbb action in host cells is unknown, however, BfUbb inhibits *in vitro* ubiquitylation and is able to covalently bind the human E1 activating enzyme. Ubiquitylation regulates a diverse number of processes essential to cellular function, including the inflammatory response, which BfUbb has the potential to modulate. Our work aims to identify further interacting partners within the ubiquitylation pathway. BfUbb may enhance the ability of *B. fragilis* to colonise the GI tract when the bacterium is in close proximity to the epithelium.

SU01Tu0900

Structural biology of Type IV secretion systems

Gabriel Waksman

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Type IV secretion (T4S) systems are molecular machines used for the transport of macromolecules across the bacterial cell envelope. T4S systems are highly versatile. Conjugative T4S systems translocate DNA from a donor to a recipient bacterium and contribute to bacterial genome plasticity, spread of antibiotic resistance or other virulence trait among bacterial pathogens. In some bacteria such as *Helicobacter pylori* (Cag PI), *Brucella suis* (VirB/D), or *Legionella pneumophila* (Dot, Icm), T4S systems are directly involved in pathogenicity as they mediate the secretion of virulence factors (DNA or toxins) into host cells. The archetypal T4S system, the VirB/D system, was defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The *A. tumefaciens* VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4). Recently, structures of large complexes formed by several of these proteins have become available shedding unprecedented light on T4S systems function.

SU01Tu0930

Modulation of membrane transport by *Legionella* Type IV effectors

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Effector proteins delivered into the cytosol of host cells modulate processes important for creating a vacuole that supports *Legionella* intracellular replication. Here we describe the biochemical function of *Legionella* effector proteins that play specific roles in controlling host membrane transport. These studies reveal new enzymatic activities and protein structures that demonstrate *Legionella* encodes novel effector proteins that can manipulate evolutionarily conserved host proteins that control membrane transport processes, which provide insight into how *Legionella* is able to construct a unique vacuole inside phagocytic host cells.

SU01Tu1000

EPEC antagonises death receptor signalling during infection

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Enteropathogenic and enterohaemorrhagic *E. coli* (EPEC and EHEC), utilise a type III secretion system (T3SS) to deliver multiple effector proteins into host cells during infection. Given their central role in the pathogenesis of many bacterial infections, elucidating the biochemical function of T3SS effectors is fundamental to understanding host-pathogen interactions. We have found that the T3SS effector NleB1 from EPEC binds to cellular death domain containing proteins and thereby inhibits death receptor signalling. NleB1 expressed ectopically or injected by the bacterial T3SS prevented Fas ligand or TNF-induced formation of the canonical death inducing signalling complex (DISC) and proteolytic activation of caspase-8, an essential step in death receptor induced apoptosis. This activity was dependent on the N-GlcNAc transferase activity of NleB1, which

specifically modified Arg117 in the death domain of FADD. The importance of the death receptor apoptotic pathway to host defence against EPEC was demonstrated using FAS-deficient mice, which showed delayed clearance of the EPEC-like mouse pathogen *Citrobacter rodentium* and reversion to virulence of an *nleB* mutant. The activity of NleB suggests that EPEC and other attaching and effacing (A/E) pathogens antagonise extrinsic cell death pathways to prevent apoptosis of infected cells, thereby interfering with a major antimicrobial host response.

SU01Tu1100

Mycobacterial Type VII secretion, what are the substrates and how do they affect virulence

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Most bacterial infections are caused by "classical" Gram-positive or Gram-negative pathogens. Notable exceptions are the pathogenic mycobacteria, such as *Mycobacterium tuberculosis*. These bacteria are characterised by the presence of unusual fatty acids known as mycolic acids, which form the main constituent of a second hydrophobic layer surrounding the cytoplasmic membrane. The presence of such a protective outer membrane is one of the main reasons why mycobacteria are tough persistent organisms, but this protective shield also poses a problem: how to secrete proteins to the cell surface or in the environment. Recently, it has become clear that the major pathway for secretion of these proteins is formed by the type VII secretion pathway, which is also known as the ESX pathway. Pathogenic mycobacteria contain up to five different of these secretion systems, three of which have been shown to be involved in bacterial growth or virulence. The number of proteins transported by this pathway is steadily increasing, which resulted in the elucidation of a consensus pattern: substrates are generally transported as folded (hetero)dimers of which the N-terminal domain forms a helix-turn-helix motif followed by a short essential secretion motif (YxxxD/E). The next challenge is to decipher the actual function of these proteins.

SU01Tu1130

Genetic determinants of outer membrane vesicle-mediated secretion

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Outer membrane vesicles (OMVs) are ubiquitously secreted by Gram-negative bacteria and are composed of a subset of outer membrane and periplasmic components. OMVs have been found to mediate the interaction of Gram-negative bacteria with their environment, including the release of virulence factors, the transfer of genetic information, the secretion of degradative factors to protect their ecological niche and acquire nutrients, as well as the nucleation of biofilms. OMVs can also play a role as an envelope stress response. Despite these critical roles, the mechanism and regulation of OMV production is still extremely cryptic. In prior genetic studies we have identified undervesiculation and hypervesiculation mutations in *E. coli*. The mutations were combined and strains analyzed for growth and vesicle production defects, envelope stability, and periplasmic content to reveal how the gene products contribute to OMV production. In addition, mutants in peptidoglycan linkage enzymes were analyzed for OMV production phenotypes and their effect on cell wall-outer membrane crosslinks. From these studies, we can conclude that OMV production is important for bacterial growth by relieving the envelope of accumulated toxic stress products, and that direct and indirect modulation of envelope crosslinking correlates with OMV production.

SU01Tu1400

Biochemical dissection of bacterial virulence and macrophage innate immunity

Feng Shao

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Many bacterial pathogens use a type III secretion system to inject virulent effector proteins into host cells. These effectors often use sophisticated biochemical strategies to manipulate host signal transduction pathway. I will present our work on three bacterial effector families, each of which defines a novel enzymatic posttranslational modification, and their functions in infection. The OspF effector from *Shigella flexneri* employs a MAPK phosphothreonine lyase activity to block host MAPK signaling and IL-8 production. The CHBP/Cif family of effectors from *Burkholderia pseudomallei* and *Enteropathogenic E. coli* (EPEC) catalyze deamidation of Gln-40 in ubiquitin and NEDD8, inactivating host ubiquitin pathway and many related cellular processes. NleE from EPEC and *Salmonella typhimurium* carries out a novel cysteine methylation modification on key ubiquitin-chain sensory proteins in host NF-κB pathway, thereby blocking NF-κB-mediated inflammatory response during infection. On the host side, the inflammasome complex is believed to be important for macrophage innate immune defense against bacterial infection. I will also discuss our recent identification and characterisation of the NAIP family of NOD-like proteins (NLRs) that serves as inflammasome receptors for bacterial flagellin and also the type III secretion apparatus, which plays an important role in restricting various bacterial infections.

SU01Tu1430

What a difference a Dalton makes: Cifs are translocated deamidases found in diverse pathogenic bacteria

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Pathogenic bacteria commonly deploy enzymes to modulate the function of host cell targets, presumably to promote virulence. Depending on the lifestyle of the bacteria, in particular during biotrophic or hemibiotrophic interactions, enzymes that catalyze subtle modifications in host cells can be important for pathogenesis and are likely to dominate. Deamidation is the irreversible conversion of the amino acids glutamine and asparagine to glutamic acid and aspartic acid respectively. Several bacterial virulence factors have evolved to catalyze deamidation of host targets, and this activity is emerging as a widespread feature in bacterial pathogenesis. One family of bacterial virulence factors that encode deamidase activity is the Cycle Inhibiting Factors (Cifs). *In vitro*, Cifs catalyse the deamidation of a specific residue (Gln40) in NEDD8 and the related protein ubiquitin. Studies both *in vitro* and in model host cells suggest that this modification interferes with recycling of neddylated cullin-RING ligases, leading to stabilisation of various cullin-RING ligases targets and preventing polyubiquitin chain formation. The structures of Cifs have been extensively studied at the molecular level, revealing adaptations to the papain-like fold that promote substrate-specific deamidation over proteolysis. Here, I will provide an overview of deamidases from bacterial pathogens, with a focus on Cifs.

SU01Tu1500

WAVE control by cooperating pathogen and host GEFs

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The WAVE regulatory complex (WRC) is crucial to host cell invasion by the bacterial pathogen *Salmonella,* which delivers virulence effectors to trigger membrane ruffling and uptake. How *Salmonella* manipulates WRC is unknown. Here, we show that Rac1 activation by the *Salmonella* Guanine nucleotide Exchange Factor (GEF) SopE was sufficient for WRC recruitment to the membrane, but not for its activation, which also required host Arf GTPase activity. Invading *Salmonella* activated and recruited Arf1 to promote membrane ruffling and uptake, and RNAi screening revealed a key role for the host Arf1 GEF ARNO in this process. ARNO triggered membrane recruitment and activation of WRC but this was dramatically enhanced when acting with SopE. *Salmonella* recruited ARNO via Arf6, which was itself recruited and activated by the host GEFs EFA6 and BRAG2. This work reveals a mechanism by which a network of pathogen and host GEFs acts to regulate WRC actin assembly and trigger invasion.

SU01Tu1600

Regulation of host innate and adaptive immune responses by *Yersinia* type III secreted effectors

James B. Bliska

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Pathogens utilise virulence factors to counteract host innate immune responses. In turn, the host can exploit virulence factors to detect pathogens and mount protective adaptive immune responses. Understanding this dual nature of virulence factors remains an important goal in the fields of microbial pathogenesis and immunology. Because many pathogens encode multiple virulence factors, an additional important goal is to determine if alteration of innate responses by one virulence factor can shape adaptive immunity to another virulence factor. To achieve these goals, we study how a prototypical type III secretion system and its cognate effector proteins in the Gram-negative bacterial pathogen Yersinia regulate murine innate and adaptive immune responses. An important role for an effector in the adaptive immune response has been established using this infection model. Mice infected with Y. pseudotuberculosis generate a dominant CD8 T cell response to a protective epitope in the effector YopE, showing that this GTPase-activating protein uniquely contributes to adaptive immunity. The presentation will describe our recent progress to toward understanding how do YopE-specific CD8 T cells protect against Y. pseudotuberculosis, what features of YopE are important for generating this response, and if other effectors enhance or decrease the CD8 T cell response to YopE.

SU01Tu1630

Studying EPEC and EHEC type III secretion system and effector proteins using *Citrobacter rodentium* infection model

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Citrobacter rodentium (CR), enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) belong to a group of extracellular Gram-negative enteric-pathogens that share the same infection strategy. They use T3SS to deliver effector proteins inside epithelial cells and induce attaching and effacing lesion to facilitate colonisation of the gut mucosa leading to extensive remodelling of the gut epithelium. EPEC and EHEC are human pathogens causing diarrheal diseases responsible for morbidity/mortality worldwide whereas CR is a natural mouse pathogen and the etiological agent of transmissible murine colonic hyperplasia. For a long time *in vivo* research of A/E pathogens was hindered by the fact that mice are resistant to EPEC/EHEC infection. Since then, infection of mice with CR has become a popular surrogate model for *in vivo* studies to understand the mechanisms of infection of clinically relevant human A/E pathogens.

In our lab we routinely use CR model to better understand EPEC/EHEC infection strategy. To enable us to follow the entire infection cycle in small animal models in real time using non-invasive techniques, we have developed an imaging facility using an IVIS Spectrum-CT. It combines computerised tomography with optical imaging, allowing the simple co-registration of the fluorescent or bioluminescent image with the CT.

SU01Tu1700

***Chlamydia* modulation of the focal adhesion kinase – a pathogenic strategy for invasion and inihibition of epithelial extrusion**

Tristan Thwaites, António T. Pedrosa, Ana T. Nogueira, REY CARABEO

Microbiology Programme, Institute of Medical Sciences, The University of Aberdeen, UK

When infected, an epithelial cell triggers an apoptotic response as part of its antimicrobial defence, alerting the neighbouring cells to initiate the process of extrusion. Some mucosal pathogens counteract this defence mechanism by translocating Type III effectors that target the focal adhesion signalling pathway. For the obligate intracellular pathogen Chlamydia, which undergoes a developmental phase when infectivity is lost, it is critical that the host cell is not extruded prematurely. We discovered that invasion of non-phagocytic cells and enhancement of cell adhesion are mediated by TarP, specifically by the mammalian-like LD motif conserved in many of the TarP homologues from a variety of chlamydial species. The TarP LD motif binds FAK to activate signalling that triggers an Arp2/3-dependent actin remodelling. At post-invasion stages, we observed the formation of unusually large focal adhesion plaques and thicker stress fibers in infected cells, which could be replicated by the overexpression of the FAK-interacting LD motif. Furthermore, TarP localised to the focal adhesions with the LD motif sufficient for this localisation. Thus, the processes of invasion, enhancement of cell adhesion, and inhibition of apoptosis are integrated into a single effector. The multi-domain nature of the virulence/survival factor TarP underscores its multifunctionality.

SU02
Fungal diseases, diagnostics and drug discovery (joint with BSMM)

SU02Mo0900

Introduction to fungal diseases and the identification of pathogenic fungi

Elizabeth M. Johnson

Mycology Reference Laboratory, Public Health England, UK

Fungi comprise the 5th Kingdom, a group of a million and a half species of amazing eukaryotic organisms covering a wealth of diverse, intricate and often beautiful forms that are neither animal nor plant. They are the major drivers of the carbon cycle comprising elite teams of detritivores without which life as we

know it could not exist. However, in the wrong place at the wrong time these opportunistic pathogens can take their role in the carbon cycle too far in attempting to return living organic matter to the soil before the host has finished with it. During this talk I will introduce the types of fungal diseases caused by yeasts and moulds encountered in the human population. These range from mild superficial infections such as dermatophyte infections and infections of the mucous membranes with *Candida* species, which are suffered by many individuals but are usually easily treated, allergic reactions due to the inhalation of fungal spores through to life-threatening invasive disease most often seen in immunocompromised patients. Identification of the causative pathogenic fungi has classically relied on careful assessment of the phenotypic characteristics of the organism but more recent developments have seen the introduction of genomic and proteomic approaches.

SU02Mo0930

Adaptive antifungal resistance mechanisms: strength in numbers

Gordon Ramage

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Fungal biofilm infections have become increasingly recognised as a significant clinical problem. One of the major reasons behind this is the impact that these have upon treatment, as antifungal therapy often fails and surgical intervention is required. This places a large financial burden on health care providers. Pathogenic fungi, such as *Candida albicans* and *Aspergillus fumigatus,* are armed with an arsenal of adaptive mechanisms that enable them to survive and proliferate within the host in the presence of antifungal agents. The aim of this presentation is to discuss the variety of adaptive resistance mechanisms used by these organisms, including extracellular matrix (ECM), efflux pump activity, extracellular DNA, stress responses and persister cells. Our recent insights into the processes and mechanisms involved in biofilm antifungal resistance will be presented.

SU02Mo1000

Offered paper **Antimicrobial peptides with antifungal activity**

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As recently discussed in Nature Reviews Microbiology 11, 146 (March 2013), "there are few public health issues of potentially greater importance for society than antibiotic resistance". Novel antibiotics are urgently needed, but the drug development pipelines are "dry". Antimicrobial peptides (AMPs), are substances that can effectively kill a broad range of life threatening multidrug-resistant bacteria and fungi. However, despite great hopes for novel AMP drugs, such drugs are still rare. To accelerate drug development we studied the connection of antibacterial activity and cytotoxicity. To accomplish this, we determined for 3,000 different 9meric peptides the concentration that killed bacteria, Rel(AB)IC75, and compared it to the concentration that lead to 75% hemolysis of human red blood cells Rel(H)IC75. Hemolytic activity was cross-validated in vitro and in vivo using cell culture and zebrafish larvae. In this process we discovered peptides that were active against Candida albicans and Aspergillus nidulans. Fluorescent labelled peptides showed that within 4 hours the peptides accumulated within the cytosol of Aspergillus nidulans, indicating an internal target.

SU02Mo1015

Offered paper **Phosphorylation of Exo84 by cyclin-dependent kinase is required for growth of *Candida albicans* hyphae**

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The exocyst is a multiprotein complex that acts to dock secretory vesicles a during the highly polarised tip growth of *Candida albicans* hyphae. We show that Exo84, one of the eight exocyst components, is specifically phosphorylated during hyphal growth. We have identified the three sites involved and have generating phosphomimetic and non-phosphorylatable substitutions singly and in combination. The triple 3A non-phosphorylatable mutant is completely unable to form hyphae whereas the 3E phosphomimetic mutant is able to form hyphae which have only mild morphological defects; thus Exo84 phosphorylation is necessary for hyphal growth. The 3A mutant also causes severe defects in yeast growth, probably because polarised growth also occurs in small buds and secondary septum formation. Interestingly, certain 1A and 2A mutants not only slow hyphal growth, but lead to a hyper-accumulation of chitin at the hyphal tip suggesting that docking of different classes of vesicle are affected. We have identified Cdc28 as the kinase. Surprisingly, Hgc1, Ccn1 and Clb2 cyclins are all required for the full pattern of phosphorylation. Finally, we show a possible physiological role of phosphorylation is to modulate the affinity of Exo84 for phospholipids.

SU02Mo1100

Recent insights into antifungal drug resistance: interactions between drugs and cells

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Fungal infections take a huge toll on the humans, animals and plants that they infect. In humans, invasive fungal infections can number in the millions and have a high mortality rate (above 40%). Treatment of fungal infections usually involves the use of one of several classes of antifungal drugs, each with a unique mechanism of action. For each of these drug classes, there is always the risk of the development of resistance. The molecular mechanisms by which cells acquire antifungal resistance have been studied in *Candida albicans*, as well as several other pathogenic fungi. These mechanisms can include changes in a) the import of the drug, b) the interactions of the drug with the target enzyme, c) the export from the cell, or d) combinations of several of these mechanisms. Particular emphasis will be paid to drug import, a long-ignored aspect of the interaction between drug and fungus. Recently, the import of sterols, which overcome the effect of several antifungal drugs, has been shown to contribute to the fungal cells' response to antifungal drugs. The future of antifungal drug development, and understanding drug resistance will be based on our current understanding of the interaction between drug and fungus.

SU02Mo1130

Chromosomal rearrangements and drug resistance

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Fluconazole is the most widely used antifungal drug because of few side effects and the ability to administer it orally. However,

resistance to fluconazole can arise rapidly in some isolates (e.g., *Marr et al. 1988*). Furthermore, 50% of fluconazole-resistant isolates carry at least one aneuploid chromosome (*Selmecki et al. 2006*) and specific aneuploidies such as isochromosome (5L) correlate very well with drug resistance, primarily because specific genes on those chromosomes confer increased resistance when present at increased copy number (*Selmecki et al. 2008*). Here we asked how aneuploidy could appear at high frequency in flucoazole-exposed cells using flow cytometry and time-lapse microscopy together with in-house image analysis pipelines. We find that exposure to fluconazole causes an uncoupling of cell cycle processes such that bud growth and cytokinesis are delayed relative to nuclear segregation, resulting in the formation of trimeras: cells with a total of 3 connected buds and 2 nuclei that divide to produce 4 daughter nuclei, two of which remain in a single bud to generate tetraploids. The tetraploids retain two spindles and often undergo daughters with unequal amounts of nuclear DNA that we interpret as aneuploids. Furthermore, trimeras are viable and survive in drug at least non-trimera cells.

SU02Mo1400

Spectrum of *Aspergillus* disease and its frequency worldwide

David W. Denning

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The genus *Aspergillus*, and its associated diseases, is extraordinary and unparalleled by any other microorganism. The spectrum of aspergillosis extends from allergy in the nose and sinuses (allergic *Aspergillus* rhinosinusitis) to the lungs (manifesting in subtly different ways) (allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS)), to slowly progressive pulmonary or sinus destruction in apparently normal individuals (and other mammals and birds) (chronic pulmonary aspergillosis and granulomatous *Aspergillus* rhinosinusitis) and to immediately life-threatening invasive infection in immunocompromised patients (invasive aspergillosis).

Globally, there are estimated to be 4.8 million with ABPA complicating asthma in adults (193 million), 6–15 million with SAFS, 1.2 million patients with chronic pulmonary aspergillosis following pulmonary tuberculosis (30-80% of the total CPA caseload, estimated at 3 million) and over 200,000 annually with invasive aspergillosis.

There is a substantial need for new antifungal agents for aspergillosis, especially oral drugs. A. terreus and A. nidulans are intrinsically resistant to amphotericin B. Acquired resistance to triazoles in *A. fumigatus* is increasing. The genomic variation in the *Aspergillus* genus is remarkable and testament to its adaptability; *A. fumigatus* and *A. nidulans* are as distantly related as fish and man representing huge evolutionary changes in ~200 million years.

Key links: www.LIFE-Worldwide.org and www.fungalinfectiontrust.org/fungaldis.html

SU02Mo1430

Immune evasion of *Aspergillus fumigatus*

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Aspergillus fumigatus can be regarded as the most important airborne fungal pathogen of patients under continuous immunosuppression. Recognition, phagocytosis and consecutive killing of conidia and hyphae by phagocytes contribute to the fungal clearance as well as to the generation of a proinflammatory immune response to trigger local infiltration of neutrophils and their migration to the site of infection. However, *A. fumigatus* has an arsenal of weapons protecting the fungus from the residual immune system in immunosuppressed patients. The 'street smart' attributes of *A. fumigatus* include the rodlet layer composed of the hydrophobin RodA covering the surface of conidia. In addition, *A. fumigatus* has some features allowing the fungus to interfere specifically with the immune response. Recent data of our laboratory showed that dihydroxy naphthalene (DHN) melanin is essential not only for inhibition of apoptosis of phagocytes by interfering with the host PI3K/Akt signaling cascade but also for effective inhibition of acidification of conidia-containing phagolysosomes. Furthermore, other compounds like gliotoxin might contribute to virulence. Also, we have carried out a number of proteomic and transcriptomic studies aiming at the characterisation of host pathogen interactions but also at the identification of antigens both for diagnosis and vaccination.

SU02Mo1500

Offered paper Investigating the potential of lipopeptides for aspergillosis therapy

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Development of safe and effective antifungals for the treatment of aspergillosis remains a significant clinical challenge. A number of microbially derived cationic lipopeptides have been identified as potent *in vitro* antimicrobial agents, but their non-cell selective mode of action, poor plasma stabilities and immunomodulatory effects have limited their potential as drug candidates *in vivo*. We have derived a wholly synthetic, first-in-class family of lipopeptides that demonstrate significant activity against *Aspergillus in vitro* and *in vivo*. Importantly these lipopeptides are not cytotoxic or haemolytic at log orders above therapeutic ranges, are plasma stable compounds, exhibit a rapid fungicidal mode of action killing germlings as well as hyphae. Visualisation of cell leakage by scanning electron microscopy and microfluidics using fluorescent markers confirmed membranes as the target and site of action of the lipopeptides. The novel lipopeptides we have engineered demonstrate early promise as a new generation of much needed antifungal therapies to address difficult to treat *Aspergillus* and other fungal infections.

SU02Mo1515

Offered paper *Aspergillus fumigatus* GNA1: fragment screening gets groovy

DEBORAH E.A. LOCKHART[1], David A. Robinson[2], Daan M.F. van Aalten[1]

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Aspergillus fumigatus may present a spectrum of clinical, diagnostic and therapeutic challenges. A new generation of antifungal agents is required to address the toxicity and emerging reports of resistance in existing therapies. The cell wall of *A. fumigatus* (*Af*) is essential for survival of the fungus and represents a drug target. Chitin, an integral component, consists of linear β(1-4) linked *N*-acetyl-D-glucosamine (GlcNAc). A new potential

antifungal target is glucosamine-6-phosphate *N*-acetyltransferase (*Af*GNA1). This enzyme *N*-acetylates glucosamine-6-phosphate to *N*-acetyl-glucosamine-6-phosphate as an intermediary step in UDP-GlcNAc biosynthesis. Fragment-based drug discovery provides a complementary and contrasting approach to traditional high-throughput methods by elaborating weakly binding small molecules. Here we assess the druggability of biotinylated *Af*GNA1 using a fragment screen based on bio-layer interferometry (Octet Red, Forte Bio). Screening the Dundee Drug Discovery Unit (DDU) fragment library gave a preliminary hit rate of 5.7% (37/652 with a response rate > 0.02 nm). A subset of seven fragments demonstrated stoichiometric binding with equilibrium dissociation constants in the micromolar range. Structural analysis of *Af*GNA1 in complex with fragment (A) illustrated the fragment binds in a groove behind the sugar substrate. Initial kinetic data suggested partial enzyme inhibition. This work suggests *Af*GNA1 may be a druggable antifungal target.

SU02Mo1600

The polysaccharides of *Aspergillus fumigatus*: not only a shield but a weapon

J.P. Latgé

Aspergillus Unit, Institut Pasteur, Paris, France

A major characteristic of all fungi is the presence of a cell wall that surrounds the cell of these eukaryotic microorganisms and protects the fungus against external stresses. The central skeleton of the fungal cell wall of the human opportunistic pathogen *Aspergillus fumigatus* is composed of chitin and branched b1,3 glucans. In addition to this fibrillar skeleton, polysaccharides that are a 1,3 glucan, galactomannan and galactosaminogalactan play the role of cement for the wall structure. These polymers are also the major component of the extracellular matrix which glues together the hyphae of a colony in vitro and in vivo. Because of their extracellular location, these polysaccharides play also an essential role during the interactions with the fungal host. During my talk, I will discuss the immune response of the host towards the constitutive cell wall polysaccharides and show that they play an active role during infection.

SU02Mo1630

Dermatophytes – from functional genomics to personalised medicine

R.J. Hay

Kings College Hospital NHS Trust, London, UK

Superficial fungal infections are the fifth most common of all human diseases and those caused by dermatophyte fungi affect people of all ages. Causes of ringworm infection dermatophytes are found in a variety of infections from scalp ringworm to onychomycosis or fungal nail disease. The former affects almost 1% of primary school children in London while the latter is found in 23% of those over 60 in northern Europe. Most infections acquired from other humans result in poor host immunological responses with evidence that recruitment of activated T lymphocytes may be poor and stimulation of cytokines from epidermal cells variable between different species. Underlying human susceptibility to severe infection has been linked to mutations in AIRE, STAT1 and STAT 9 genes. Studies of the early stages of human infection have shown changes in production of a variety of proteases, such as subtilisins, regulated in part by the presence of amino acids. While diagnosis still relies on direct microscopy and culture new molecular based diagnostic tests are proving highly useful although patients are still screened by microscopy. Treatment is often driven by protocols backed by evidence-based clinical trial data but for some infections treatment is individualised to include combinations of therapies. For instance we have seen the replacement of griseofulvin as the sole oral agent with a choice of antifungals such as terbinafine, itraconazole and fluconazole. Treatment regimens have also altered with an increasing emphasis on shorter daily treatments (terbinafine), intermittent therapies given for a week (itraconazole) or weekly single doses of drugs (fluconazole); in topical therapy the main change has been reduction of treatment lengths from one month to a single application, for instance, in the case of terbinafine, a film forming solution in tinea pedis. To improve on existing treatments there has been the wider use of combinations of drugs eg itraconazole and terbinafine or drugs and surgical approaches including the use of laser nail treatment This range of choice opens the possibility of providing individualised treatments depending on the patient, the organism and the extent and type of the infection.

SU02Tu0900

***Cryptococcus*: clinical disease, diagnostics, and drug discovery and optimal use**

Thomas S. Harrison

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Cryptococcus species have become one of the leading fungal causes of disease and death worldwide. *C. neoformans* has been estimated to cause up to a million infections per year and is associated with 15–20% of all AIDS-related deaths in parts of Sub-Saharan Africa. Additionally, the emergence of a hypervirulent lineage of *C. gattii* in British Columbia in 1999 has demonstrated the threat posed by *Cryptococcus* sp. in regions outside their usual range and to immunocompetent hosts.

A new point-of-care immunodiagnostic test has the potential to facilitate screening and pre-emptive treatment as a cost-effective preventative strategy in patients with late-stage HIV infection in areas of high incidence, as well as enabling earlier, primary care-based, diagnosis for all symptomatic cases. While drug discovery aimed specifically at *Cryptococcus* species is very limited, optimising our use of current antifungal drugs, including in resource-limited settings, together with improved diagnosis, has the potential to significantly reduce the global disease burden..

SU02Tu0930

The capsule of *Cryptococcus neoformans*

Arturo Casadevall

Albert Einstein College of Medicine, USA

Cryptococcus neoformans has the distinction of being the only encapsulated human pathogenic fungus. The capsule is composed of polysaccharide and is a major virulence factor. The major component of the capsule is glucuronoxylomannan (GXM). During infection the polysaccharide capsule of *C. neoformans* can undergo dramatic enlargement. Although capsules are critically important virulence factors for many pathogenic microorganisms very little is known about their architecture because they are highly hydrated structures that are vulnerable to dehydration. Hence, we have applied several new techniques to gain insight into the structure of the cryptococcal capsule. Using a combination of dynamic and static light scattering we were able to deduce that the capsule grows by elongation of single molecules and that GXM is a branched structure that appears to be an oligodendrite. By employing molecular tweezers we have explored the mechanical properties of the capsule in the presence and absence of capsule-binding antibodies. The results show that the binding of protective antibodies, but not non-protective antibodies, results in major changes into the visco-elastic properties of the capsule. Finally, we have been able to image the growth of the capsule by light microscope and analysis of the rate of growth has provided new insights into capsule assembly.

SU02Tu1000

Offered paper **Understanding the route of *Cryptococcus* uptake by macrophages**

JENSON LIM, Robin May

University of Birmingham, Birmingham, UK

Cryptococcus is a fatal fungal pathogen that causes cryptococcosis and is a leading cause of death in immunocompromised individuals, particularly AIDS patients. *Cryptococcus* is an opportunistic and facultative intracellular human pathogen, which is not only resistant to phagocytosis, but is able to survive and proliferate within the mature phagolysosome following uptake. From there, *Cryptococcus* can either lyse the host cell or move between macrophages via a non-lytic process called vomocytosis. Interestingly, although much is known about the opsonisation and uptake of *Cryptococcus* via opsonic receptors (e.g. complement or Fc receptors), little is known about the involvement of non-opsonic receptors in cryptococcosis.

Here we use a combination of inhibitors, bone marrow macrophages from knockout mice and ectopically overexpressed non-opsonic receptors to dissect the role of non-opsonic receptors in driving phagocytosis of this important pathogen. We show that, despite the presence of a thick mannan-based capsule, non-opsonic uptake of *Cryptococcus* is dependent on the dectin family of receptors but not the mannose receptor. Together, this suggests that phagocytosis of cryptococci *in vivo* is likely to depend on a complex interplay between different signalling pathways, which likely has significant implications for the intracellular behaviour of this pathogen.

SU02Tu1015

Offered paper **Drug redeployment: a high-throughput screening approach to identify compounds that inhibit microbial intracellular proliferation**

REBECCA HALL, Kerstin Voelz, Robin May

University of Birmingham, Birmingham, UK

Infection is a continuous battle between host immune defences and the invading pathogen. Many microbes have evolved strategies to evade phagocytosis, thus promoting their survival within the host, and increasing the possibility of establishing an infection. One such mechanism is the ability to proliferate within the macrophage phagosome. Intracellular proliferation has been observed for many fungal and bacterial species, and enables the pathogen to remain dormant inside the macrophage and establish a latent infection. *Cryptococcus neoformans* and *C. gattii* are fatal human pathogens infecting both immune suppressed and immune competent individuals, respectively. The virulence of these pathogens is associated with their intracellular proliferation rate (IPR). Therefore, compounds that reduce the IPR may hold promise as novel therapeutic agents. Using a genetically engineered strain of *C. neoformans,* which ubiquitously expresses GFP, we have developed an intracellular proliferation assay to quantify the IPR of *C. neoformans* in murine macrophages. High throughput screening of a library of FDA approved compounds identified several compounds that significantly inhibited *C. neoformans* intracellular proliferation. We are now working to determine the efficacy and mode of action of these agents in order to test whether they are also likely to be effective against other intracellular proliferating pathogens.

SU02Tu1100

Candidiasis today: epidemiologic and therapeutic landscape

Michael A. Pfaller

JMI Laboratories, North Liberty, Iowa, USA; University of Iowa College of Medicine, Iowa City, Iowa, USA

The epidemiology of invasive candidiasis ((IC) and candidemia has been described in numerous single-center, sentinel and population-based surveys conducted throughout the world. However, the dynamic nature of trends in IC in the U.S. suggests that this issue still merits considerable attention. Despite the fact that *C. albicans* remains the most common species causing IC worldwide, the incidence of *C. albicans* bloodstream infections (BSIs) in the U.S. was found to have decreased in population-based surveys conducted in 2008-2011 versus those in 1992-1993. In its stead both *C. glabrata* and *C. parapsilosis* have emerged as important and potentially antifungal-resistant pathogens. The species-specific incidence of *C. parapsilosis* doubled and that of *C. glabrata* increased by more than four-fold between 1992-1993 and 2008-2011. Data from the SENTRY Antifungal Surveillance Program found that in the US, the frequency of *C. glabrata* as a cause of BSIs increased from 18% in 1992-2001 to 25% in 2001-2007, with a concomitant increase in fluconazole resistance from 9% to 14%. The emergence of co-resistance to both azole and echinocandin antifungal agents is another concern with this species of *Candida*. Similarly the decreased susceptibility of *C. parapsilosis* to the echinocandins is a concern with this species. The emergence of less-common and antifungal-resistant species such as *C. guilliermondii, C. rugosa, C. haemulonii* and *C. auris* is a further issue that has been recognised with the broader application of molecular and proteomic methods of identification. Given the importance of local and regional epidemiology in the selection of early and appropriate antifungal therapy in patients with IC, continued surveillance is warranted.

SU02Tu1130

***Candida* diagnostics**

Darius Armstrong-James

Imperial College London, UK

There have been a number of advances in the diagnosis of invasive candidiasis over the last decade that have the potential to greatly enhance our ability to rapidly detect and treat infection appropriately. This is crucial, because late diagnosis leads to increased mortality. Furthermore, newer approaches such as MALDI-TOF raise the possibility that earlier detection of drug-resistant strains may also be possible, which will also likely impact on patient outcome. In this talk I will review the standard as well as recent approaches to Candida diagnostics that are available, including those in development, and their likely clinical impact.

SU02Tu1400

Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*

Yasin F. Dagdas, Lauren S. Ryder, Michael J. Kershaw, Yogesh Gupta, George Littlejohn, NICHOLAS J. TALBOT

School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter, UK Email: N.J.Talbot@exeter.ac.uk

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* forms a specialised infection structure called an appressorium. The infection cell generates enormous turgor, which is focused as mechanical force to breach the rice cuticle and facilitate entry of the fungus into plant tissue. A hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium which allows re-establishment of polarised fungal growth. Re-modeling of F-actin at the appressorium pore is necessary for cortical rigidification and localisation of proteins associated with membrane curvature to the point of plant infection. Septin-mediated cytoskeletal re-modeling is required

for development of a penetration peg that ruptures the host cuticle and leads to invasion of epidermal cells by biotrophic invasive hyphae of *M. oryzae*. Septin-mediated plant infection is controlled by NADPH oxidase activity and a regulated burst of reactive oxygen species occurs within the appressorium. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. We will also describe the potential operation of a pressure-mediated checkpoint, mediated by a cell wall mechanosensor protein, that is necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.

SU02Tu1430

One step ahead? The evolution of fungicide resistance in *Septoria* leaf blotch of wheat

JOHN A. LUCAS, Hans J. Cools, Bart A. Fraaije

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Fungicides play an important role in cereal production in Europe, safeguarding yield and quality in high disease seasons. Septoria leaf blotch, caused by the fungus *Mycosphaerella graminicola*, is the most important foliar disease of wheat. The pathogen has developed resistance to several classes of single site fungicides, including the methyl-benzimidazoles (MBCs), the quinone-outside inhibitors (QoIs) and most recently the azoles. The loss of efficacy of the MBCs and QoIs was associated with selection of mutations causing single amino acid substitutions in the fungicide target proteins, β-tubulin and mitochondrial cytochrome *b* respectively. These changes conferred high levels of resistance without any apparent fitness cost; hence the mutations have persisted in the fungal population even in the absence of fungicide use. The emergence of resistance to azole fungicides has followed a different pattern, with gradual accumulation of mutations and promoter changes in the sterol 14α-demethylase encoding gene (*CYP51*). This process has led to gradual shifts in the sensitivity of the fungal population, associated with the emergence of progressively more complex *CYP51* genotypes. The implications of these developments for management of the disease will be discussed.

SU02Tu1500

Offered paper **Characterising putative secreted proteins of the microsporidian *Nosema ceranae***

GRAHAM THOMAS, James Cresswell, Ken Haynes

University of Exeter, Devon, UK

Microsporidia are obligate intracellular eukaryotic parasites related to fungi, possessing greatly reduced genomic and cellular components. The microsporidian *Nosema ceranae* threatens the two economically important pollinators, honey (*Apis mellifera*) and bumble (*Bombus* species) bees and has been causally linked to colony collapse disorder. Nosemosis has a complex epidemiology affected by host, pathogen and environmental factors. Although a draft of the *N. ceranae* genome has been published, the molecular basis underpinning pathogenicity is not known. We hypothesise effectors essential to disease progression exist amongst *N. ceranae* secretome genes. In this study we have started characterising these genes using Gateway® cloning technology and identify candidate effectors by their expression in *Saccharomyces cerevisiae*. We offer experimental data supporting the identities of NcORF-01664 and NcORF-01663 as polar tube proteins (PTP) 1 and 2 respectively and have identified a putative Ptp4. Future studies will focus on functional characterisation of effector candidates. Increased knowledge on disease progression will ultimately lead to disease mitigation.

SU02Tu1515

Offered paper **Live-cell imaging and analysis of the modes-of-action of a new generation of small, synthetic antifungal peptides**

Alberto Munoz[1], Akira Alexander[1], Dilip Shah[2], Jose Marcos[3], NICK READ[1]

[1]University of Edinburgh, Edinburgh, UK, [2]Donald Danforth Plant Science Center, St. Louis, USA, [3]IATA – CSIC, Valencia, Spain

We are actively exploring the use of synthetic antifungal peptides (AFPs) as novel pharmaceuticals to treat human fungal infections. Our recent studies have focused on the mode-of-action of various small rationally designed AFPs and synthetic AFPs derived from plant defensins. For this purpose we are using the fungal model Neurospora crassa and the human pathogen Aspergillus fumigatus combined with live-cell imaging of fluorescently labelled AFPs and other live-cell probes, inhibitor treatments and mutant analyses. PAF26 is a de novo-designed hexapeptide possessing two well-defined motifs: N-terminal cationic and C-terminal hydrophobic regions. We have characterised how each motif is responsible for PAF26's dynamic antifungal mechanism of action involving the electrostatic interaction with cells, cellular internalisation, and cell killing. PAF26 increases cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) and several Ca^{2+} signalling/homeostatic mutants are resistant to the AFP. Different peptide sequences derived from the γ-core motifs of MsDef1 and MtDef4 defensins inhibit conidial germination and hyphal fusion, and influence $[Ca^{2+}]_c$, with different potencies and specificities. Our results provide new mechanistic insights into the mode-of-action of AFPs that should help us design new synthetic AFP-based drugs with improved activity and stability.

SU02Tu1600

Privileged drug-like libraries for biological evaluation and an introduction to SMSdrug.net

JOHN SPENCER, MARK C. BAGLEY

Dept of Chemistry, University of Sussex, Falmer, East Sussex, UK

A short presentation on the role of SMSdrug.net, an MRC-BBSRC-EPSRC joint initiative for bringing together chemists and biologists to collaborate on drug discovery, will be given (MCB). This will be followed by a short account on the types of molecules (libraries) made in the Spencer laboratory, of potential use to microbiologists for testing and as chemical tools for biological research. The libraries consist of a number of privileged scaffolds including benzodiazepines, oxazoles, pyrimidines and benzotriazepines.

SU02Tu1615

Offered paper **PI3K/Akt/mTOR signaling mediates protection of oral epithelial cells from *Candida albicans*-induced damage**

DAVID MOYES[1], Celia Murciano[2], Chengguo Shen[1], Jonathan Richardson[1], Stephen Challacombe[1], Julian Naglik[1]

[1]King's College, London, London, UK, [2]University of Valencia, Valencia, Spain

The ability of epithelial cells to discriminate between commensal and pathogenic microbes is essential for healthy living. Key to these interactions are mucosal epithelial responses to pathogen-induced damage. Using reconstituted oral epithelium we assessed epithelial gene transcriptional responses to C. albicans infection by microarray. Signal pathway activation was monitored by Western blot and TransAM assay and their role in C. albicans-induced damage protection determined using chemical inhibitors.

Transcript profiling demonstrated early up-regulation of epithelial genes involved in immune responses. Many of these genes constituted components of signaling pathways, but only NF-κB, MAPK and PI3K/Akt pathways were functionally activated. We demonstrate that NF-κB and MAPK signaling are independent of PI3K/Akt signaling, and that PI3K/Akt signaling plays a key role in epithelial immune activation and damage protection via mTOR activation and GSK3β deactivation. Thus, PI3K/AKT signaling plays a critical role in protecting epithelial cells from damage during mucosal fungal infections.

SU02Tu1630

Offered paper **Functional characterisation of *Candida glabrata* ORFs with no orthologue in *Saccharomyces cerevisiae***

LAUREN AMES, Jane Usher, Ken Haynes

University of Exeter, Exeter, UK

Candida glabrata is a significant and increasingly common pathogen of humans yet its mechanism of virulence remains unclear. Comparative genomic studies revealed that *C. glabrata* is more closely related to the non-pathogenic yeast *Saccharomyces cerevisiae* and that both these genomes are distinct from *C. albicans*.

In order to explore *C. glabrata* virulence attributes, a barcoded *C. glabrata* deletion library was generated targeting ORFs with no orthologue in *S. cerevisiae*. To gain insight into the function of these ORFs, mutants were phenotypically screened on over 60 conditions targeting a range of cellular processes and structures. Mutants were also tested for infection-related properties including biofilm formation, antifungal agent susceptibility and finally for virulence in a *Drosophila melanogaster* infection model. As such, novel phenotypes associated with the deletion of previously uncharacterised ORFs were uncovered.

ORFs with notable phenotypes were taken forward for further characterisation. An adapted genome-wide synthetic genetic interaction approach was used to create genetic interaction networks for *C. glabrata* ORFs over-expressed in *S. cerevisiae*. A *C. glabrata* chromatin remodeler showed genetic interactions with genes involved in metal ion homeostasis and DNA damage repair. Further experimentation has proven this approach successful for dissecting the roles of *C. glabrata* ORFs.

SU02Tu1645

Offered paper **The *Candida albicans* ORFeome project: towards a genome-wide overexpression strain collection**

KEUNSOOK LEE[1], Mélanie Legrand[2], Yogesh Chaudhari[1], Laurence Arbogast[2], Sophie Bachellier-Bassi[2], Murielle Chauvel[2], Vitor Cabral[2], Audrey Nesseir[2], Irena Pelinska[1], Emmanuelle Permal[2], Ute Zeidler[2], Sadri Znaidi[2], Louise Walker[1], Carol Munro[1], Christophe d'Enfert[2]

[1]Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK, [2]Institut Pasteur, Unité Biologie et Pathogénicité Fongiques / INRA USC2019, Paris, France

Candida albicans, the most common human fungal pathogen, has 6215 predicted ORFs; however 74% of them remain uncharacterised. To study gene functions and improve our understanding of pathogenicity, the gene deletion or knock-out method has been broadly used. However, this method has certain disadvantages due to recessive lethal mutations and the diploid nature of *C. albicans*. Furthermore, functional compensations of paralogous genes and multigene families have been observed in many single gene null mutants, which could impact on the presentation of substantial phenotypes. Therefore, an alternative strategy has been successfully applied, to other species, which is to create over-expression gene libraries and screen for gene functions and other applications. The *C. albicans* ORFeome project is creating three new resources for the *Candida* community, including (1) a *C. albicans* ORFeome library, (2) a library of bar-coded *C. albicans* over-expression vectors, and (3) a library of *C. albicans* over-expression strains. All three libraries will be available to the community upon completion. These will advance the research field of genome-wide over-expression screens, identification of antifungal drug targets, the development of libraries for protein localisation, protein complex identification, *in vivo* virulence studies, and other applications, which will also enrich our understanding of pathogenicity.

SU03
Impact of bacteriophage in the environment

SU03Mo0900

Structure and assembly of bacteriophage

Michael G. Rossmann

Dept of Biological Sciences, Purdue University, West Lafayette, IN 47908, USA

All bacteriophages in the World's oceans taken together are the largest amount of bio-mass on Earth. A large proportion of these phages have a dsDNA genome an icosahedral head and a tail. The heads protect the genome whereas the tails are highly efficient machines for inserting the genome into the bacteria across membranes, cell walls and periplasmic space. Mechanisms will be described by which the empty proheads are packaged with their genome for phages T4 and phi29. The mechanism will also be described by which the contractile tail of T4 ejects its genome. The assembly of the tail-less ssDNA phiX174 will also be described, including the assembly of the tail when needed at the time of infection.

SU03Mo0930

Stx phages: where are they and why are they there?

Heather E. Allison

School of Biological Sciences, University of Liverpool, BioSciences Building, Crown Street, Liverpool, UK

Shortly after the first *E. coli* O157:H7 outbreak in 1982 it was discovered that a lambdoid phage was responsible for the ability of its *E. coli* host to produce Shiga toxin. Today we find that Stx phages are still driving the dissemination of shigatoxigenic potential and continuing to expand their host range. The presence of Stx phages on a farm environment has been explored using both culture dependent and independent methods. The impact of Stx phages on their bacterial host has been measured using a variety of modern molecular methods and postgenomic strategies including genome comparisons, RNASeq and reporter gene assays. These data provide an interesting insight into the prevalence of Stx phages, novel phage regulatory mechanisms, adsorption targets and novel gene function. The results indicate that there is still much to learn about the basic biology of temperate phages and their complex adaptation to the lifestyles of their bacterial hosts.

SU03Mo1000

Exploring *Clostridium difficile* phages and its CRISPR system

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Technology, Atlanta, GA, USA; [3]Microbial Pathogenesis Laboratory, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK
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The major bacterial pathogen *Clostridium difficile* causes antibiotic associated diarrheal disease which impacts significantly on human morbidity and mortality. In other systems, bacteriophages contribute to bacterial virulence, population dynamics and genome evolution. To determine the potential impact of phage infection on *C. difficile* we have modelled phage–host interactions and analysed the CRISPR system which is a form of adaptive immunity that bacteria use to resist phage infection.

Our results suggest that the impact of phage infection in this system is widespread and multi-layered. Modelling the host range data has shown significant nested and modular interactions which suggests that multiple routes of co-evolution occur between phages and hosts.

Modular patterns of phage infection are generally seen in species with active CRISPR systems and our analysis of the *C. difficile* CRISPR system target a diverse set of phage sequences. Fittingly, the CRISPR profiles generated are consistent with the host-range results that were determined experimentally.

One of the striking features of the *C. difficile* CRISPR system is that in addition to the bacterial encoded CRISPR arrays, several *C. difficile* prophages encode multiple and diverse CRISPR arrays which target other phages. This suggests an intriguing novel mechanism for prophages to block secondary phage infection.

SU03Mo1100

Offered paper **Three novel configurations of CRISPR/Cas systems and unique evolutionary histories of *Corynebacterium diphtheriae* strains**

VARTUL SANGAL[1], Peter Fineran[2], Paul Hoskisson[1]

[1]University of Strathclyde, Glasgow, UK, [2]University of Otago, Dunedin, New Zealand

CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR associated proteins) systems protect bacteria against invading nucleic acids by recognising and destroying them in a multistep cascade. We compared 17 Corynebacterium diphtheriae genomes and identified three CRISPR/Cas systems, variants of Type II or Type I-E systems. The Type II system lacked cse2 or cas4 genes that are involved in spacer acquisition. This system was replaced by a variant of Type I-E (I-E-a) in 6/17 isolates where the repeat array was inserted between cas3 and cse1 genes. Three isolates possessed an additional Type I-E variant (I-E-b) with cas genes arranged as two divergent operons. These systems are present on genomic islands and are flanked by mobile genetic elements. A phylogenetic incongruence of the core genome with palindromic repeats and cas1 gene and the difference in the G+C contents between CRISPR/Cas systems indicate three independent horizontal acquisitions of these systems. Some spacers showed similarities with bacteriophages of different species, indicating the presence of broad host range corynebacteriophages. However, most spacers lacked identity with phage or plasmid sequences, suggesting an unexplored reservoir of corynebacterial bacteriophages and plasmids. The spacer diversity in conjunction with the core genomic phylogeny reveals unique evolutionary histories of C. diphtheriae strains.

SU03Mo1115

Offered paper **Characterising the impact of temperate bacterial viruses of *Pseudomonas aeruginosa* isolated from cystic fibrosis patient**

FRANCESCA EVEREST[1], Mohammad Tariq[1], Anthony De Soyza[2], Audrey Perry[2], John Perry[2], Stephen Cummings[1], Clare Lanyon[1], Darren Smith[1]

[1]University of Northumbria, Newcastle Upon Tyne, UK, [2]Freeman Hospital, Newcastle Upon Tyne, UK

Cystic Fibrosis (CF) is a common autosomal recessive genetic mutation in Caucasian populations. A hallmark feature is bronchial inflammation and the production of mucus, so offering a microenvironment suitable for opportunistic bacterial pathogens to colonise e.g. *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus, Haemophilus influenza* and the *Burkholderia cepacia* complex. Chronic infection leads to restricted lung function that is progressive and can be life threatening. Temperate bacterial viruses (bacteriophages) encode moron genes that aid bacterial survival, they can offer selective genetic traits that may increase antimicrobial resistance and in some instances, are shown to evade the complement cascade. For these reasons temperate phages play a role in bacterial infection and microbial evolution in the chronically infected lung. This study indicates that physiological and clinical pressures drive phage-host evolution within this micro-environment. Selective pressure leads to alteration in the phages infectivity or cellular target and that inducible phages from CF PA isolates have co-evolved with this bacterial background. It was found that phages chemically induced from a CF PA had higher levels of infectivity and wider host ranges, when compared to phages from other chronic lung conditions (for example non-CF bronchiectasis). These results support an evolutionary difference between phages from similar pathophysiological conditions.

SU03Mo1130

Bacterial control of bacteriophage predation by toxin–antitoxin-mediated suicide

GEORGE SALMOND

Department of Biochemistry, University of Cambridge, UK

As the most abundant biological entities on Earth, bacterial viruses outnumber their hosts by ten to one. Nevertheless, the bacteria continue to survive despite predation by their viruses. They achieve this through adaptations in a molecular "arms race" that defines the predator-prey relationships seen in virus-host interactions. Some phage-resistance mechanisms operate post-infection. One category is the abortive infection (Abi) systems. We have studied an Abi system that is bifunctional; it acts as both a Toxin-Antitoxin (TA) system and an antiviral system. This defines the "Type III" TA system based on a toxic protein that is inhibited by a small RNA pseudoknot antitoxin. Infection by some phages leads to activation of the toxin – and death. From an evolutionary perspective this event might be viewed as an altruistic suicide by the bacterial host as it terminates propagation of the invading viral predator in a clonal bacterial population, thereby saving sibling bacteria. Phages evolve various mechanisms to escape the potentially lethal effects of the Abi system. One mechanism is through RNA-based molecular mimicry that suppresses lethality in the bacterium and enables viral replication and dissemination.

SU03Mo1400

Speaker to be confirmed

SU03Mo1430

Offered paper **Characterisation of a novel set of environmental bacteriophages which infect *Burkholderia pseudomallei***

JINYU SHAN[1], Jiraporn Gatedee[2], Sunee Korbsrisate[2], Edd Galyov[1], Martha Clokie[1]

[1]Department of Infection, Immunity, and Inflammation, University of Leicester, Leicester, UK, [2]2Department of Immunity, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

Burkholderia pseudomallei has a natural habitat in the tropical soil

environment where it is exposed to a number of biotic factors including bacteriophages, which have not been fully recognised. We have recently isolated over thirty environmental phages from different geographical locations in North-Eastern Thailand. Most of the newly isolated phages belong to the Podoviridae and can infect both *B. pseudomallei* and *B. thailandensis*. They have similar morphology, genome sizes and genomic arrangement. They encode integrases from the family of tyrosine recombinases and therefore may have an access to the lysogenic lifecycle. Furthermore, the genomes also encode a MazG like protein, which may act to reduce the cellular level of guanosine 3',5'-bispyrophosphate (ppGpp), an important regulatory molecule in the bacterial stringent response. MazG homologues have been identified in several other phages including *Burkholderia cenocepacia* and *Pseudomonas aeruginosa* as well as in cyanophages. It has been suggested that MazG-encoding phages may play an active role in nutrient starved bacteria. Thus our research on these *Burkholderia* phages indicates that they are likely to be a significant factor in the environmental biology and virulence of *B. pseudomallei*.

SU03Mo1445

Offered paper **Characterisation of a novel freshwater cyanopodophage**

SIOBHAN WATKINS, Joy Watts, James Smith, Paul Hayes

University of Portsmouth, Portsmouth, UK

Cyanophages are known to influence population dynamics (diversity and scale) and functional activity of cyanobacteria in the sea, however, we know much less about interactions between cyanophages and their hosts in freshwater. Cyanophages were isolated from freshwater habitats known to support cyanobacterial blooms. One isolate, designated MHI42, was selected for further study. Morphologically MHI42 appears to be a member of the *Podoviridae*, but with a lager capsid in comparison to other members of the same family. It was not possible to generate PCR amplicons from host lysates using primer sets designed to amplify genomic sequences from other cyanophages, which is consistent with this isolate being novel. MHI42 was shown to have a broad host range and was able to infect and produce lysis in some strains of *Microcystis* and *Planktothrix*: other host strains from the same genera seemed to be resistant to infection or they entered what appeared to be a chronically infected state. This cyanopodophage seems to have a novel genome and to be able to vary its life cycle, infecting a range of hosts. MHI42 may act as a useful model for the examination of host range, and, ultimately, gene transfer across broad taxonomic divides, in the environment.

SU03Mo1500

Actinophage–host interactions

Maggie Smith

University of York, Department of Biology, York, UK

Bacteria in the phylum *Actinobacteria* comprise some of the most feared human and animal pathogens, highly valued producers of antibiotics and other bioactive natural products as well as major plant and animal symbionts, bioremediators and probiotic agents. This physiological diversity is matched by the morphological diversity of the *Actinobacteria*, which can be cocciod, rod-coccoid, rods, fragmented hyphae or permanently mycelial with a highly differentiated sporulation stage. Actinophages are likely to be as diverse as their hosts needing to adapt to the specific features of individual host genera such as the *Mycobacterium* cell wall or the complex morphological life style of *Streptomyces*. Most sequenced actinophage genomes are those that infect *Mycobacterium*, *Proprionibacterium* and *Streptomyces* and we can compare the nature of the phage populations. Some specific adaptations will be described that appear to have evolved in *Streptomyces* phages.

SU03Mo1600

***Flavobacterium* phage genomics and dynamics**

Karin Holmfeldt

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Viruses are fundamental to environmental ecosystems, yet our ability to study them is bottlenecked by the lack of ecologically-relevant isolates. This has resulted in 'unknowns' dominating culture-independent surveys and that ecological functionalities are mainly derived from experiments on human associated *Escherichia* phages. Through work with phages infecting the aquatic bacterium *Cellulophaga baltica* (phylum *Bacteroidetes*; family *Flavobacteriaceae*) 12 novel phage genera could be delineated from 31 sequenced phages, with represents from 4 known families (*Myo-*, *Podo-*, *Sipho-*, and *Mivroviridae*) as well as a novel large non-tailed, icosahedral ssDNA phage. While this diversity is consistent with other non-aquatic heterophage collections (e.g., *Escherichia* and *Pseudomonas* phages) it contrasts previously sequenced marine phage collections (e.g. cyanophages). However, unlike cyanophages, the *Cellulophaga* phages shared very few genes with non-aquatic phages. Insight into the phages genetic functions opened up for the possibility of different replication strategies among the different phages, both concerning host specificity and lytic/lysogenic infection patterns. Experimental data supported these hypotheses and highlighted the importance of host strain choice for phage infection strategies. Together this much needed diverse and novel model system provides novel identification for metagenomic studies, and provides an environmentally-relevant phage-host experimental platform for studies of ecologically important phage-host interactions.

SU03Mo1630

***Campylobacter* phage population dynamics in chickens**

I.F. Connerton

School of Biosciences, Division of Food Sciences, University of Nottingham, UK

Controlling campylobacters in poultry represents one of the greatest challenges to the agriculture and food industries if they are to achieve consumer and governmental demands to reduce human food borne disease. Control of *Campylobacter* is an obvious target for phage therapy because a large proportion of poultry reared for meat harbour these organisms as a part of their intestinal flora with few practical alternatives for reduction. However, simply introducing bacteriophages to chickens is unlikely to result in the elimination the target bacteria, since like most predators phage seldom eliminate their prey in nature. Treatments that do not eliminate but reduce the numbers of campylobacters below critical thresholds will benefit public health. Our studies to investigate the sustainable use of bacteriophages have revealed some important aspects of *Campylobacter* ecology in response to phage predation. The recovery of campylobacters from biofilms after phage treatment identified cultures in which otherwise virulent phage had established a relationship with their hosts typical of a poorly understood phenomenon referred to as the carrier state life cycle (CSLC). In CSLC cultures bacteria and bacteriophages remain associated in equilibrium and represents an important ecological sink for *Campylobacter* bacteriophage.

SU03Mo1700

The ubiquitous phage: a model virus for school laboratory classes

JOANNA VERRAN[1], James Redfern[1], Daniel Burdass[2]

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UK and Society for General Microbiology, UK; [2]Society for General Microbiology, Marlborough House, Basingstoke Road, Spencers Wood, Reading, UK

The school curriculum makes frequent reference to viruses in terms of structure, infections, epidemiology etc., but it is not likely that the teacher will be able to demonstrate principles of practical virology in the laboratory. The aim of this project was to develop effective, safe and reproducible laboratory exercises that demonstrated aspects of virology using bacteriophage.

Phage are the most common organism on the planet, and are key to many global and local processes (for example, keeping cholera pandemics in check). In the classroom, the use of phage and host enables safe demonstration of infection kinetics and host-cell lysis.

For each practical exercise in the 'phage resource', information for students, teachers and technicians is provided, along with illustrations, general information, model data, reference to other laboratory activities and supplier contacts. Each exercise is mapped to activities noted on the appropriate specification. The five practical exercises are: total and infectious phage count; phage inactivation using acidified ethanol (*Escherichia coli* and T4); virus diagnosis using PCR ('HSV-1' or 'VZV'); prevention of yoghurt production using *Streptococcus thermophilus* and dairy phage.

The presentation will describe rationale, development, trialling and preliminary evaluation of the resource in advance of launch, and subsequent evaluation of impact and learning.

SU03Tu0900

Ecological stoichiometry drives rapid co-evolution of bacteria and phage

JAY T. LENNON, Megan L. Larsen

Indiana University, Department of Biology, 1001 E. 3rd Street, Bloomington, IN 47405, USA

Ecological stoichiometry is a theoretical framework that considers the balance of elements (e.g., nitrogen and phosphorus) between organisms and their environment. This theory has successfully been used to explain various ecological phenomena, including life history strategies, the outcome of species interactions, and ecosystem processes. Recently, it has been suggested that stoichiometry may also influence the rates of evolution, but evidence for this hypothesis is lacking. We conducted a long-term experiment that examined the eco-evolutionary dynamics of marine *Synechococcus* (Cyanobacteria) and a lytic phage under nitrogen-limited (low N:P) and phosphorus-limited (high N:P) conditions. Nutrient stoichiometry had strong effects on the temporal dynamics and stability of the microbial communities, which coincided with rapid phenotypic changes between viruses and their hosts. Whole genome sequencing of isolates revealed that nitrogen-limited hosts had mutations in the regulatory pathways for lipopolysaccharide (LPS) biosynthesis, the viral receptor used for infection, while phosphorus-limited hosts had structural mutations associated with LPS. Together, our results reveal that nutrient stoichiometry is a bottom-up driver that can generate rapid eco-evolutionary feedback in microbial food webs.

SU03Tu0930

Bacteria–phage co-evolution in natural environments

Britt Koskella

University of Exeter, Cornwall Campus, Exeter, UK

The dynamic microbial communities of long-lived hosts will change over time due to immigration of new species, interaction with the host immune system, and selection by bacteriophage viruses (phages), but the relative roles of each process are unclear. Previous metagenomic approaches confirm the presence of phages infecting host microbiota and experimental coevolution of bacteria and phage populations in the laboratory has demonstrated rapid reciprocal change over time. The key challenge is to determine whether phages influence these bacterial communities in nature, in the face of other selection pressures. I used a tree-bacteria-phage system to measure reciprocal changes in phage infectivity and bacterial resistance within microbial communities of tree hosts over one season. My combined data on phage adaptation to bacterial hosts over space and time provides clear evidence for both phage-mediated selection on bacterial communities and bacterial-mediated selection on phage communities in nature. These local adaptations and reciprocal changes suggest that phages indeed play a key role in shaping the microbiota of their eukaryotic hosts.

SU03Tu1000

Abiotic and biotic impacts on bacteria–phage relationships

MARTHA R.J. CLOKIE[1], Sachia J. Traving[2], Mathias Middelboe[2], Jinyu Shan[1], Fatima Vukusic[1], Sunee Korbsrisate[3], Ed Galyov[1]

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The infection dynamics between bacteria and their bacteriophages are strongly influenced by biotic and abiotic factors. For example, the latent period, burst size, lifecycle and the proportion of bacterial cells that are killed can all change according to physiological state of their host bacteria, and to physical parameters such as temperature and pH. I will present three examples of how the bacteria-phage relationship changes in response to different variables. 1) Using *Synechococcus* WH7803 as a model marine cyanobacterium I will show that decreasing culture pH, lengthens the eclipse period but decreases the latent period and the resulting phage burst size. This work has implications in our understanding of how ocean acidification will impact cyanobacterial primary productivity. 2) To give an example of the host dynamics impacting phage dynamics I will show how the relationship between *Clostridium difficile* and bacteriophages differs when the bacteria are grown on epithelial cells compared to growth under in vitro conditions. These observations demonstrate the importance of applying relevant physiological conditions to laboratory studies of phages and are relevant to the study of phages for use in therapeutic conditions. 3) Finally, I will present evidence in *Burkholderia spp* (*B. pseudomallei* and *B. thailandensis*) that demonstrates how the temperature in which bacterial cells and phages are incubated can directly impact the lifecycle of phages. When exposed to their hosts at 37 or 42 °C the phages to follow a lytic life cycle but they lysogenise them when incubated at 23 °C. This apparent temperature dependent lysogeny can explain the widespread distribution of genetically similar phages over 100 km scales in NE Thailand. The *Burkholderia* phages all encode integrases and have not been described before, either as isolated phages, or as part of the substantial sequencing effort for this organism. I will explain the environmental and potential medical implications of this observation.

SU03Tu1100

Differential cyanophage gene expression in response to light

Richard Puxty[2], David Evans[2], David Scanlan[2], ANDREW MILLARD[1]

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Cyanobacterial cells of the genera *Prochlorococcus* and *Synechococcus* dominate in the open ocean environment, their co-occuring cyanophages are equally abundant. The cyanophage S-PM2 was the first phage found to carry the "photosynthetic" genes *psbA* and *psbD,* which encode for the core PSII proteins PsbA and PsbD respectively. Both of these proteins are rapidly turned over during photosynthesis and if not replaced can lead to photoinhibition. Cyanophage are thought to maintain photosynthesis by the expression of phage *psbA* and *psbD* during the infection cycle. The *psbA* gene of S-PM2 is unusual in that it contains a self splicing group I intron. Our previous work has also shown the presence of a non-coding RNA , *CfrI,* that is antisense to *psbA*.

We present data on the response of phage encoded genes under high and low light. With increased expression of both phage *psbA* and *CfrI* under high light. Additionally, there are differences in the pool of *psbA* pre-mRNA transcripts, caused by the differential splicing of the intron within *psbA*. With evidence the intron splicing is regulated by *CfrI*, which can bind to pre-mRNAs and prevent splicing. Thus, providing a mechanism to respond to changes in environmental light conditions.

SU03Tu1130

Use of aqua-phage in fish farming

Hans Petter Kleppen

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Bacteriophages are ubiquitous in aquatic environments and are known to have great influence on the bacterial composition of these. This is also true for bacteriophages infecting bacteria with relevance to fish farming. Lysogenic bacteriophages are often carriers of virulence genes in pathogenic bacteria, and lytic bacteriophages have been linked to the natural clearance of bacteria at the end of disease outbreaks. Excessive use of antibiotics and the increasing emergence of resistant bacteria have called for novel approaches to treatment of bacterial disease in aquaculture. One such alternative is the therapeutic use of bacteriophages. ACD Pharmaceuticals have been working with phages for therapeutic application in aquaculture for nearly three years. In this talk we present our view on phage therapy applied in fish farming and what we have learned from our endeavors.

SU04
Sir Howard Dalton Young Microbiologist of the Year Competition

SU04Tu0910

Modulation of enhancer looping and differential gene targeting by Epstein–Barr virus transcription factors directs epigenetic reprogramming

MICHAEL J. McCLELLAN[1], C. David Wood[1], Opeoluwa Ojeniyi[1], Tim J. Cooper[1], Aditi Kanhere[2,3], Aaron Arvey[4], Helen M. Webb[1], Richard D. Palermo[1,5], Marie L. Harth-Hertle[6], Bettina Kempkes[6], Richard G. Jenner[2], Michelle J. West[1]

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Epstein–Barr virus (EBV) epigenetically reprogrammes host B-lymphocytes creating immortal cells to facilitate viral persistence. Host-cell transcription is deregulated principally through the cooperative actions of EBV EBNA 2, 3A, 3B and 3C, with cellular genes deregulated by unique EBNA 3 subsets through largely unknown mechanisms. Importantly we have elucidated the mechanism of gene targeting by specific EBNA 3 family members demonstrating that this is driven by differential binding of EBNA 3A, 3B or 3C to regulatory elements in both a gene and cell-type specific manner. Strikingly, we also detect extensive targeting of common sites by EBNA 2 and 3 proteins, predominantly in long-range enhancers. Investigating shared sites at the novel targets *WEE1* and *CTBP2* we have demonstrated that EBNA 3 proteins repress transcription by modulating enhancer-promoter loop formation establishing repressive chromatin hubs or preventing active hub assembly. Re-ChIP analysis revealed that EBNA 3 proteins do not bind shared sites simultaneously with EBNA 2 but compete for binding thus modulating enhancer-promoter interactions. At a unique intergenic EBNA 3A and 3C binding site at the *ADAM28/ADAMDEC1* locus we found that enhancer-promoter looping directed epigenetic repression of both genes. Our findings provide a paradigm for host-cell reprogramming through modulation of enhancer-promoter interactions.

SU04Tu0925

Bacterial lipoteichoic acid interacts with the human platelet membrane receptor CD36

ALYSON MURRAY, Jennifer Mitchell

UCD School of Biomolecular and Biomedical Science, University College Dublin, Ireland

Infective Endocarditis (IE) is a microbial infection of the endocardial surface of the heart. The interaction and adhesion of gram positive bacteria with platelets plays an essential role in the initiation of IE. *Staphylococcus aureus* and *Streptococcus mitis* are gram positive bacteria that are leading causes of IE. In this study we show that Lipoteichoic acid (LTA) on the surface of these bacteria interacts with platelets. This was demonstrated using direct binding assays blocking with free LTA and antibodies to LTA. We show that a *S. aureus* ltaS deletion mutant that does not express LTA exhibits a decreased level of binding to platelets. This binding is restored in an isogenic ltaS complemented strain. CD36 is an 88kDa glycoprotein of the scavenger receptor B family that has been shown to act as a co-receptor for LTA with TLR-2 in the immune recognition of LTA. We propose that CD36 on the surface of platelets is acting as a receptor for *S. mitis* and *S. aureus*. We identified the minimum binding region of CD36 to which the LTA of *S. mitis* and *S. aureus* interacts with as amino acid 118-182 with two lysine residues 164 and 166 essential for binding. This study has shown for the first time the interaction of bacterial LTA with CD36 on the surface of platelets and identified the amino acid region of CD36 to which LTA binds.

SU04Tu0940

Genomic analysis of bioactive-producing *Bacillus subtilis* marine sponge isolates

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New antimicrobial compounds are urgently needed to combat the spread of multi-drug resistant pathogens, but few new options are entering the drug discovery pipelines for clinical trials. Our goal is to exploit the biotechnological potential of

spore forming bacteria from untapped sources, such as deep sea marine sponges, as a new source of novel antimicrobials.

The power of coupling functional based assays with genomic approaches has enabled us to identify a novel class I lantibiotic, subtilomycin, which is active against several clinically relevant pathogens. Subtilomycin is encoded in the genomes of eight different *B. subtilis* strains from coastal and deep sea marine sponges, which cluster together phylogenetically and form a distinct group in comparison to other wild and domesticated *B. subtilis* strains. In addition to its obvious clinical relevance, the presence of subtilomycin and other putative bioactive gene clusters may be providing these native strains with a specific competitive advantage(s) within the stringent confines of the marine sponge environment.

We have demonstrated that the industrial and biotechnological potential of this new group of marine sponge isolates, which can produce a cocktail of bioactive compounds, is being unlocked by the application of "integrated biodiscovery genomics". Furthermore our results highlight the importance of mining unique environments and habitats for new lead compounds with potential therapeutic applications.

SU04Tu0955

ATP-dependent formation of viral ribonucleoprotein particles in Kaposi's sarcoma associated herpesvirus: a target for small molecule inhibitors?

SOPHIE SCHUMANN, Adrian Whitehouse

Faculty of Biological Sciences and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic virus responsible for the occurrence of Kaposi's sarcoma, primary effusion lymphoma and some types of multicentric Castleman's disease. The virus replicates in the nucleus of the host cell and requires cellular export factors to export viral mRNAs in order to allow efficient translation of viral genes in the cytoplasm. However, while mammalian mRNA export is linked to splicing, the majority of KSHV mRNAs are intronless, prompting the virus to circumvent this step. KSHV therefore encodes ORF57, a protein which interacts with the human transcription/export (hTREX) complex to form an export competent ribonucleoprotein particle, which facilitates nuclear export of viral mRNA. In this study we show that formation of the ORF57-mediated ribonucleoprotein particle is ATP-dependent, which presents a novel antiviral target. Our results suggest ATP-cycle dependent remodelling of the hTREX complex, which affects the ability of ORF57 to recruit the endogenous complex. Following these findings we present a mechanism for disruption of the ORF57/hTREX interaction. Using virtual high-throughput screening we identified compounds which could be used to specifically inhibit viral ribonucleoprotein particles and as a consequence disrupt virus lytic replication, while allowing endogenous protein complex formation.

SU04Tu1030

Control of a bacterial toxin by an antitoxic RNA pseudoknot

Francesca Short

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Bacteria are constantly threatened by their viral parasites, the bacteriophages, and have evolved an array of resistance mechanisms to protect themselves. One such mechanism involves the altruistic suicide of infected cells before the phage can replicate, and this can be mediated by the ToxIN system of *Pectobacterium atrosepticum*. $ToxIN_{Pa}$ is a Type III toxin-antitoxin system that comprises a toxic ribonuclease ($ToxN_{Pa}$) and an antitoxic RNA ($ToxI_{Pa}$), which together form an inactive complex. Phage infection triggers the release of $ToxN_{Pa}$, thereby killing the cell and protecting the clonal population. The success of this antiviral strategy therefore depends on the very strong inhibition of $ToxN_{Pa}$ by the RNA, $ToxI_{Pa}$, under normal conditions. In this study we aimed to define how the ToxI RNA recognises and inhibits its toxin partner.

We show, through cross-inhibition experiments with a second ToxIN system ($ToxIN_{Bt}$ from *Bacillus thuringiensis*), that ToxI RNAs are highly selective inhibitors. Processed and unprocessed forms of $ToxI_{Pa}$ can inhibit $ToxN_{Pa}$ in vitro, without any cellular factors or exogenous energy. This inhibition is linked to the self-assembly of a trimeric $ToxIN_{Pa}$ complex, previously observed by crystallography. We explain the basis for ToxI antitoxin selectivity through the crystal structure of the $ToxIN_{Bt}$ complex. Finally, we explore the biological role of $ToxIN_{Bt}$. This system does not appear to mediate phage resistance, unlike $ToxIN_{Pa}$, but does promote plasmid retention during both vegetative growth and sporulation. Our results present a picture of ToxIN as an addictive, self-assembling molecular machine, which can drive distinct adaptive advantages in different populations of bacterial hosts.

SU04Tu1045

RbpA, a novel transcriptional activator in *Streptomyces coelicolor* A3

ALINE TABIB-SALAZAR[1], Richard A. Lewis[1], Bing Liu[2], Steve J. Matthews[2], Mark S. Paget[1]

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RbpA is an RNA polymerase (RNAP)-binding protein that stimulates transcription in actinobacteria, including *Streptomyces coelicolor* and *Mycobacterium tuberculosis*. RbpA specifically activates the vegetative form of RNAP as opposed to alternative forms of the enzyme. We explain this specificity by showing that RbpA binds directly to the principal vegetative sigma subunit but not to more diverged, alternative sigma factors. Genetic and biochemical approaches were used to identify the sigma and RbpA domains involved in this interaction and RbpA mutants were isolated that are impeded in transcriptional activation. The structures of RbpA from *S. coelicolor* and *M. tuberculosis* RbpA will also be presented.

SU05
Pathogen genomics – current clinical applications

SU05Tu1400

Rapid whole-genome sequencing improves diagnostic and public health microbiology

Sharon J. Peacock

Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Wellcome Trust Sanger Institute, UK; Health Protection Agency, UK

The latest generation of benchtop DNA sequencing platforms can provide an accurate whole genome sequence (WGS) for a broad range of bacteria in less than a day. These could be employed in routine diagnostic practice to more effectively contain the spread of multidrug resistant pathogens. This talk will focus on the opportunities that rapid microbial WGS presents for the investigation of nosocomial outbreaks caused by multidrug resistant bacteria, and the identification of genetic determinants of antimicrobial resistance associated with a stratified medicine approach to patient care.

SU05Tu1430

To understand local epidemiology you must first understand the global

Simon R. Harris

Wellcome Trust Sanger Institute, Hinxton, UK

The decreasing cost and turnaround time of whole genome sequencing (WGS) provides huge potential for use in the clinical setting. It could both simplify typing of infectious diseases and provide far greater resolution for detection of transmission and outbreak investigations. However, clinical sequencing data must be interpreted with knowledge of the characteristics of the particular pathogen. Assessment of transmission requires knowledge of the rate at which mutations would be expected to accrue, and outbreaks can only be confirmed with knowledge of the background diversity. The only way to attain such knowledge is to sample broadly, both temporally and spatially, to provide a historical and global context within which recent clinical isolates can be placed. Recently, WGS was used to confirm an outbreak of MRSA on a special care baby unit in Cambridge, UK. Analysis of the outbreak isolates in isolation identified that they were a single-locus variant of the dominant UK hospital clone, EMRSA15, and provided clinically-relevant information about antimicrobial resistance and virulence genes carried. However, the addition of a global dataset provided the context to show that the outbreak was not part of the hospital-associated EMRSA15 expansion, but rather a community-associated lineage most similar to isolates of Indian origin.

SU05Tu1500

***Clostridium difficile* – a global evolutionary experiment played out in our hospitals**

Brendan Wren

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The driving force for the development of next generation sequencing has been the requirement for SNP analysis of human genomes. Applying this technology to the smaller genomes of bacterial pathogens has provided unprecedented opportunities to study mutational change and natural variation of these infectious agents. This has further provided new information on the transmission tracking, evolution and pathogenesis of bacteria. The lecture will illustrate where whole genome sequencing has been used to establish the global phylogeny of the hospital acquired infection *Clostridium difficile,* and will demonstrate the spread and transmission tracking of the 017 and 027 hypervirulent lineages at regional, national and global levels. The lecture will discuss the genetic and functional differences that may explain how and why these lineages dominate and how this information can potentially be applied in disease prevention, as well as studying the evolution and pathogenesis of this problematic pathogen.

SU05Tu1600

Offered paper **PATHSEEK: automated, whole-genome sequencing of pathogens: potential applications for public health microbiology**

AMANDA BROWN[1], Dan Depledge[2], Jolyon Holdstock[1], Katja Einer-Jensen[3], Martin Shutten[4], Judy Breuer[2]

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Recent advances in whole genome sequencing (WGS) have resulted in reduced cost coupled with reduced turnaround time, and for the first time WGS can be considered a viable technology for a range of applications, from clinical diagnostics and resistance monitoring to public health and epidemiology. PATHSEEK to deliver in 24-48h whole genome information on the pathogen, including all possible drug resistance mutations, as well as data on nosocomial infections, from one patient sample in one single assay. PATHSEEK will revolutionise clinical diagnostics and patient management by delivering all the data required for truly personalised treatment, allowing for both better care and a reduction in the use of non-effective antimicrobials. In turn this will help to reduce the emergence of drug-resistance and minimise the unnecessary extra medical expenses from the use of inappropriate drugs. PATHSEEK will also allow for increased public health management of nosocomial infections, allowing for the identification of community clusters and transmission events, which on a larger scale can permit European and WHO reference centres to follow in real time the spread of infection, and allow for earlier interventions to mitigate pandemics. PATHSEEK is funded under the European Commission Seventh Framework Programme, grant number 304875, Theme Health.2012.2.3.0-1

SU05Tu1615

Offered paper **Developing a pipeline for identifying candidates for use in pathotyping**

KATE HOWELL[1], Lucy Weinert[1], Roy Chaudhuri[1], Shi-Lu Luan[1], Sarah Peters[1], Brendan Wren[3], Paul Langford[2], Andrew Rycroft[4], Andrew Tucker[1], Duncan Maskell[1]

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Pathotyping assays are the future of clinical diagnostics and surveillance, but finding candidates for this approach typically requires years of clinical and experimental data. We describe a pipeline to go from whole genome sequencing data to candidates for use in molecular diagnostic tests. We have collected over 200 isolates of the pig pathogen Haemophilus parasuis, from various clinical backgrounds and sequenced their genomes. This bacterium is responsible for a spectrum of disease states, ranging from non-clinical carriage through to respiratory and systemic disease and the availability of detailed clinical data allows a sophisticated analysis of associations between particular genomic features and particular disease outcomes (Aragon *et al.*, 2010). The pipeline includes gene prediction software to extract coding sequences, homology group analyses and regression analysis. These data can form the basis for defining core and accessory genomes from which the presence and absence of genes and associations of genomic features with disease state can be analysed, leading to a shortlist of pathotyping candidates.

Aragon, V., Cerdà-Cuéllar, M., Fraile, L., Mombarg, M., Nofrarías, M., Olvera, A., Sibila, M., *et al.* (2010). Correlation between clinico-pathological outcome and typing of *Haemophilus parasuis* field strains. *Veterinary Microbiology*, **142(3-4)**, 387–393. doi:10.1016/j.vetmic.2009.10.025

SU05Tu1630

Rapid and deep genome sequencing of virus outbreaks and epidemics

Paul Kellam

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

Virus infections still represent one of the largest global causes of mortality and morbidity. Virus genome sequences are today a prerequisite for understanding the molecular basis of viral

replication, pathogenesis recently epidemiology. Next generation sequencing now forms the cornerstone of the next leap in understanding how genetic changes in virus genomes and host cell genomes influence the biological properties of viral pathogenesis, transmission and host susceptibility to infection. We are now experiencing the third virus outbreak in 4 years all of which have been informed by rapid whole virus genome sequence analysis. Starting with the 2009 pandemic influenza A H1N1 (2009) which swept around the world and whilst not as pathogenic as previous pandemics, has caused in excess of 14000 deaths we have moved on now to Influenza A H7N9 in China. Meanwhile an outbreak of a new coronavirus MERS-CoV has infected 90 people causing 45 deaths. In this talk I will discuss the progress that has been made in harnessing next generation sequencing for virology during these outbreaks and epidemics and some of the challenges ahead.

SU05We0900

Inflammation-inflicted enterobacterial blooms: a hotspot for horizontal gene transfer and colicin-dependent competition

Lubov Nedialkova, Rémy Denzler, Martin B. Koeppel, Manuel Diehl, Diana Ring, Roman G. Gerlach, BÄRBEL STECHER

LMU Munich, Max von Pettenkofer-Institut, München, Germany

The mammalian gut harbors a dense microbial community interacting in multiple ways, including horizontal gene transfer (HGT). Using a mouse colitis model, we found that *Salmonella*-inflicted enteropathy elicits parallel blooms of the pathogen and of resident commensal *Escherichia coli*. These enterobacterial blooms boosted conjugative HGT of a colicin-plasmid (pCollb) from *Salmonella enterica* serovar Typhimurium (*S.* Tm) to *E. coli* at unprecedented rates [1]. Moreover, we showed recently that the environmental conditions in inflammation-inflicted enterobacterial blooms favour colicin-dependent competition of *S.* Tm and *E. coli*. Colicin Ib (CollB) produced by *S.* Tm kills sensitive *E. coli* strains (e.g. MG1655) *in vitro*. Production of CollB conferred a competitive advantage to *S.* Tm over sensitive *E. coli* MG1655 in the inflamed gut. In contrast, an avirulent *S.* Tm mutant strain defective in triggering gut inflammation did not benefit from CollB, which was due to the absence of inflammation. Expression of CollB is regulated by iron limitation and the SOS-response. In addition, CirA, the cognate outer membrane receptor of CollB on colicin-sensitive *E. coli*, is induced upon iron limitation. We conclude that growth in inflammation-induced blooms favours expression of both, *S.* Tm CollB and the receptor CirA, thereby fuelling CollB dependent competition of *S.* Tm and commensal *E. coli* in the gut.

1. Stecher B, Denzler R, Maier L, Bernet F, Sanders MJ, *et al.* (2012) Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. Proc Natl Acad Sci U S A 109: 1269-1274.

SU05We0930

Emergence and global spread of epidemic *Clostridium difficile*: linking pathogen genotype to disease phenotype

Trevor D. Lawley

Bacterial Pathogenesis Laboratory, Wellcome Trust Sanger Institute, Hinxton, UK

Epidemic *Clostridium difficile* (027/BI/NAP1) rapidly emerged in the past decade as the leading cause of antibiotic-associated diarrhea in healthcare facilities worldwide. Using whole-genome sequencing and phylogenetic analysis, we defined the global population structure of *C. difficile* 027 allowing us to identify the key genetic changes linked to its emergence and trace the patterns of global spread. Using a murine infection model we show that mice infected with epidemic *C. difficile* 027, but not other human virulent variants, develop chronic intestinal disease and pathological imbalances in the intestinal microbiota (termed dysbiosis) that was refractory to vancomycin treatment leading to relapsing disease. In contrast, treatment of *C. difficile* 027 infected mice with feces from healthy mice rapidly restored a diverse, healthy microbiota and resolved *C. difficile* disease. We used this model to identify a simple mixture of six phylogenetically diverse intestinal bacteria, including novel species, which can re-establish a health-associated microbiota and clear *C. difficile* 027 infection from mice. Thus, we demonstrate that epidemic *C. difficile* 027 effectively induces intestinal dysbiosis as a mechanism of disease causation, and outline a rational approach to harness the therapeutic potential of health-associated microbial communities to potentially treat *C. difficile* disease in humans.

SU05We1000

Understanding polymicrobial communities and disease progression

KENNETH D. BRUCE[1], Lucas R. Hoffman[2,3], Mary P. Carroll[4], Geraint B. Rogers[5]

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Whilst our conventional understanding of infection has relied on single species models, the detection of multiple microbes in many infectious diseases is increasingly reported. Whilst there are many clinical examples of polymicrobial infections, those affecting the airways often prove particularly challenging to treat. The first step in the search for improved treatments is defining the composition of the microbiota present by culture-independent analysis. Studies focusing on the bacterial species present using next generation sequencing have revealed many microbes not previously reported in airways infections. For example, sequencing studies have identified many species of anaerobic bacteria in cystic fibrosis airway secretions not identified by routine culture methods. Studies are now defining the degree to which the composition and distribution of the species detected vary at different stages of individual infections. For example, in chronic airways infections, longitudinal studies are now defining how the mix of species in an individual varies over time with changes in symptoms, disease severity, and antibiotic therapy. Overall though, much remains to be understood about these complex interacting communities and their relation to the host. Increasing our understanding of this community may offer fresh treatment insights to slow or ideally prevent disease progression.

SU05We1100

Offered paper **Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for hospital acquisition of *Staphylococcus aureus***

JAMES PRICE[1], Tanya Golubchik[2], Kevin Cole[3], Daniel Wilson[4,5], Derrick Crook[4,6], Guy Thwaites[7], Rory Bowden[5], A Sarah Walker[4,6], Timothy Peto[4,6], John Paul[1,3], Martin Llewelyn[1,8]

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Background We employed whole-genome sequencing (WGS) to investigate patient-to-patient transmission of *Staphylococcus aureus* on an adult intensive care unit in a non-outbreak situation. We compared the utility of WGS with conventional methods (*spa*-typing plus epidemiological information).

Methods Screening swab isolates (n=276) collected over a 14-month period were *spa*-typed and underwent WGS to investigate their relatedness at high resolution. Admission details were recorded for all patients.

Findings 43 *S. aureus* acquisitions (either new positive status or change of genotype) were detected during the study period. WGS of available isolates identified only 6/37 (16%) acquisitions as patient-to-patient transmissions. *spa*-typing and overlapping patient stay falsely identified 3 patient-to-patient transmissions (all MRSA) and failed to detect one acquisition and four transmissions (2 MRSA).

Interpretation In a non-outbreak situation patient-to-patient transmission explains only a minority of new acquisitions, suggesting the possibility of other sources. Transmission events suggested by *spa*-typing can be disproved using the high resolution offered by WGS. Otherwise unsuspected transmission events that lack obvious temporal association are revealed by WGS. Our understanding of *S. aureus* epidemiology will be redefined by WGS.

SU05We1115

Offered paper **Antibiotic resistance prediction in *Staphylococcus aureus* – linking genome sequence to phenotype**

SANDRA REUTER[1], Matthew Holden[1], Simon Harris[1], Stephen Bentley[1,2], Rosy Reynolds[3], Nicholas Brown[4], Andreas Karas[4], Kathy Raven[5], Elizabeth Blane[5], Julian Parkhill[1], Estee Torok[2,5], Sharon Peacock[2,5]

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Bacterial whole genome sequencing will become increasingly used during outbreak investigations, and the information generated could be mined to provide rapid prediction of antibiotic resistance patterns. We sought to predict resistance patterns of a UK collection of 1,800 *Staphylococcus aureus* bacteraemia isolates.

We examined isolates from the British Society of Antimicrobial Chemotherapy (BSAC) Resistance Surveillance Project, tested previously by the BSAC agar dilution method for a range of 18 antibiotics. Additional isolates from 3 hospitals in the East of England were tested using the Vitek-2 platform. To determine resistance profiles arising through gene acquisition, we compiled a list of known determinants to search the assembled genomes. For resistances arising from mutations in core housekeeping genes sequence reads were mapped against a reference molecule of sensitive alleles to identify characterised SNPs bestowing resistance. Genetic predictions were performed blinded to phenotypic susceptibility data.

Overall, 98.8% of all predictions for susceptibility or resistance were correct. Discrepancies relate to acquired genes like *ermC*, which are carried on plasmids that can be lost in culture.

Automated protocols are increasing in accuracy for predicting resistance phenotype from genotype, but novel mechanisms of resistance continue to emerge. This must be considered and targeted phenotypic testing performed alongside.

SU05We1130

Predicting drug resistance from sequence: applications in tuberculosis and gonorrhoea

Philip D. Butcher

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An area of great importance in healthcare to which genomics may now be applied is antibiotic resistance. Rapid genotypic testing for antibiotic susceptibility is now feasible, based on whole genome sequencing (WGS), new detection technologies and accurately predictive phenotype-genotype correlations. Tuberculosis and gonorrhoea are paradigms for how genomics can have a direct impact on clinical care. Treatment for MDR-TB and XDR-TB is prolonged (18 months), requiring cocktails of antibiotics, initially given empirically prior to susceptibility tests that can take >4 weeks. Direct WGS of *M.tuberculosis* from a specimen or after short term culture can predict antibiotic susceptibility, thereby informing antibiotic choice and resulting in reductions to treatment times, risk of onward transmission and healthcare budgets. Increasing antibiotic resistance prevalence rates for *Neisseria gonorrhoeae* threaten current empirical treatments. Rapid genotypic tests predicting antibiotic susceptibility at point of care allow a personalised approach to antibiotic selection, sparing the use of the third generation cephalosporins in favour of effective antibiotics, such as penicillin, that remain useful in most cases, but which are precluded from empirical use by the >5% resistance prevalence rates in the community. Genomics thus provides opportunities for enhanced antibiotic stewardship with direct benefit to public health.

SU05We1300

***Mycoplasma amphoriforme* – on the track of a new respiratory pathogen**

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The diagnosis of mycoplasma infection in clinical practice poses difficulties because of their fastidious growth. *Mycoplasma amphoriforme* was first isolated from sputum of a PID patient suffering chronic bronchitis of unexplained origin. The slow growth we determine, 17.4 h, may have helped this organism to evade detection. A qPCR-based assay in sequential specimens from chronically infected patients showed fluctuating bacterial loads with periods of no detection. WGS analysis showed clonality of the infection in a single host, had chronic remitting character and evidence of transmission between patients. Mutations conferring resistance were identified in isolates from patients undergoing multiple antibiotic treatment. WGS of the original isolate and available isolates from the UK, France and North Africa show that *M. amphoriforme* is freely recombining and the gene pool is global.

Further epidemiological studies of samples from routine respiratory virus screening revealed the presence of *M. amphoriforme* in 23 patients from South-East Scotland. There are ongoing studies in South East Asia where MAM has been detected in LRTI samples.

The tools now available show that qPCR and WGS have a crucial role in tracking a novel pathogen and are the prelude for research to understand the biology and transmission of *M. amphoriforme*.

SU05We1330

Offered paper **Whole-genome sequencing for national surveillance of Shiga-toxin-producing *Escherichia coli* O157:H7**

PHILIP ASHTON[1], Tim Dallman[1], Claire Jenkins[1], Liljana Petrovska[2], John Wain[3], Kathie Grant[1]

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Objective Shiga toxin producing Escherichia coli (STEC) serotype O157 is an important cause of gastrointestinal infection in humans which can result in severe clinical outcomes including haemolytic ureamic syndrome (HUS) and death. Phage susceptibility and MLVA are the molecular typing methods currently used by the UK reference laboratories to investigate the molecular epidemiology of O157:H7 and therefore to inform national surveillance practices.

Methods Over 500 STEC O157:H7 strains isolated between 1990-2012, from outbreak and sporadic cases were selected for WGS. Sequenced reads for each isolate were aligned against the E. coli O157:H7 Sakai genome (NC_002695.1). Single nucleotide polymorphisms (SNPs) in the core genome were identified and phylogentic methods applied.

Results More than 5000 SNPs were identified. Strains that were epidemiologically linked clustered phylogenetically and distinctly from sporadic cases. Certain strains with no apparent epidemiological association were phylogenetically linked. Subsequent epidemiological investigations revealed previously obscure links indicating potential cryptic and sustained outbreaks.

Discussion Whole genome sequencing of STEC O157:H7 provides unprecedented clarity in identifying linked cases. Furthermore we show evidence that WGS can allow us to detect more ambiguous associations such as the recurrence of environmental contamination and distributed food network contamination that are beyond the capacity of current typing methods.

SU05We1345

Offered paper **Using bacterial whole-genome sequencing to investigate the spread of *Mycobacterium bovis***

HANNAH TREWBY[1], Roman Biek[1], David Wright[3], Tom Mallon[2], Stanley McDowell[2], Carl McCormick[2], Anthony O'Hare[1], Richard Orton[1], Robin Skuce[2], Rowland Kao[1]

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Current sequencing technologies enable the study of population processes at unprecedented genetic resolution. Here, we describe how bacterial whole genome sequencing provides new insights into the persistence and spread of an important veterinary bacterial pathogen.

Bovine tuberculosis (bTB) is caused by the bacterium *Mycobacterium bovis*. It is primarily a disease of cattle, but in Britain and Ireland failure to eradicate bTB in the cattle population has been linked to infection in the Eurasian badger. Despite extensive research effort, the exact roles of badgers and cattle in bTB in the Britain and Ireland remain unclear and controversial.

Using isolates from Northern Ireland, we sequenced all samples available for a recently emerged *M. bovis* molecular type (n=146, including six badger samples). Combining these molecular data with detailed demographic information on the cattle population, we investigate the fine-scale processes involved in transmission of the pathogen, untangling the relative roles played by movements of infected cattle between farms compared to local spatial processes. Our results also show minimal divergence among *M. bovis* genomes isolated from cattle and badgers, implying recent transmission between the two species.

SU05We1400

TB transmission networks

Tim Peto

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The Modernising Molecular Microbiology Consortium have collected over 1000 isolates of Mycobacterium Tuberculosis obtained from patients in the West Midlands and Oxfordshire in the UK over 6 years. We aimed to establish a methodology to reliably infer transmission networks. Whole genome sequencing with an Illumina platform was used and sequences were assembled by reference mapping. The errors in sequencing was measured. Isolates were accompanied with meta-data including the time and place of isolation, the clinical course of the TB and data on the social setting of the patients. From this genomic based networks were compared with social networks allowing calibration of the genomic networks. Initial results from the West Midlands and Oxfordshire showed that transmission networks, as well as sporadic cases of TB, could be easily distinguished. The potential problem of missing cases will be discussed. In order for this methodology to be used for routine public health purposes, a suitable database and software solution allowing automated analysis and reporting of the data needs to developed and tested in national pilot studies. The current phase of development will be discussed.

SU05We1500

Population genomics of post-vaccine changes in pneumococcal epidemiology

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The 7-valent conjugate polysaccharide vaccine, targeting the pneumococcal capsule, was introduced in the USA in 2000, resulting in a significant net fall in the rate of pneumococcal disease. This was a consequence of a very substantial decline in the rates of infection caused by bacteria with vaccine-type capsules being partially offset by an increase in disease associated with pneumococci with non-vaccine-type capsules. In order to ascertain the population dynamics underlying this alteration, whole-genome sequencing was used to characterise 616 *Streptococcus pneumoniae* isolates obtained through surveillance of the asymptomatically carried population in Massachusetts between 2000 and 2007. This revealed that extensive serotype replacement had occurred largely through the emergence of genotypes relatively closely related to those that had dominated before the vaccine's introduction. Comparisons within individual

lineages showed the role of transformation in facilitating capsule switching to non-vaccine serotypes, and the emergence of drug resistance, although such recombination occurred at significantly different rates across the species. These alterations resulted in little overall effect on accessory genome composition at the population level, contrasting with the decrease in pneumococcal disease rates after the vaccine's introduction. Hence genome-based surveillance promises to inform our understanding of the epidemiology and biology of even genetically diverse pathogens.

SU05We1530

Offered paper **Taking control: overcoming technical biases in metagenomics**

DENISE M. O' SULLIVAN[1], Sasithon Temisak[1], Thomas Laver[2], Gavin Nixon[1], Ken Laing[3], Nicholas Redshaw[1], Philip D. Butcher[3], David J. Studholme[2], Carole Foy[1], Jim F. Huggett[1]

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Metagenomics provides an opportunity to comprehensively understand the microbial population of any given environment, ranging from soil or water to the human gut. The most exciting technological leap that has contributed to this field has been the development of NGS, with the latest technologies producing up to 150 million sequence reads, allowing microbial community profiling in unprecedented depth.

With recent increased activity in metagenomics, there is an ever-greater need for the availability of control materials for analysis. Such material facilitates both the qualitative and quantitative evaluation of the impacts of sample processing, sequencing methods and data analysis on findings, as well as aiding in the comparison of different studies.

We have developed a metagenomic control material comprising genomic DNA from a panel of 10 common human bacterial pathogens. Species are represented at differing abundances. We used this material to evaluate aspects of 16S ribosomal DNA amplicon sequencing, comparing observed proportions of each species to our assigned values. Different approaches, including library preparation, primer specificity and 16S variable region, were investigated and found to influence the quantitative and qualitative data. These findings suggest the application of control materials could provide an invaluable tool to evaluate technical performance when conducting metagenomics experiments.

SU05We1545

Offered paper **Variation in complex surface polysaccharide biosynthetic loci in *Acinetobacter baumannii***

JOHANNA KENYON[1], Kathryn Holt[2], Derek Pickard[3], Gordon Dougan[3], Ruth Hall[1]

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Extracellular polysaccharides are major immunogenic components of the bacterial cell envelope. However, little is known about the genetics of their biosynthesis in *Acinetobacter baumannii*, an important nosocomial pathogen. Draft genome sequences of more than 220 multiply antibiotic resistant *A. baumannii* isolated in Australia between 1996 and 2011 were used to identify two regions associated with surface polysaccharides. A gene encoding an O-antigen ligase was not found, indicating that lipopolysaccharide (includes O antigen) is not produced. Thus, the loci are likely involved in capsule and lipooligosaccharide outer-core (OC) synthesis. Twenty distinct capsule loci and 8 OC loci were identified. When publicly available complete and draft genomes were included, these numbers increased to 54 capsule and 15 OC loci. The capsule loci contained genes for activated sugar synthesis, glycosyltransfer, modification, repeat-unit processing and export. The OC loci contained mainly glycosyltransferase genes. Extensive variation was observed in the two global clones, GC1 and GC2, that include the majority of multiply antibiotic resistant isolates. Overall, 8 capsule and 5 OC loci were identified in GC1 strains, and 15 capsule and 3 OC loci in GC2. Capsule and OC variability and associated antigenic switching may contribute to the success of these clones.

SU05We1600

Host-restriction, the processes underlying the microevolution and success of *Streptococcus equi*

Andrew Waller

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Strangles, caused by *S. equi*, is the most frequently diagnosed infectious disease of horses worldwide. However, the global diversity and mechanisms underlying its evolution as a host-restricted pathogen remain unknown. Here we define the global population structure of *S. equi* and chart the emergence of a new epidemic lineage that has spread across Europe. Our data reveal a dynamic genome that continues to mutate, decay, amplify and acquire genetic material. Convergent deletion of the 'equibactin' locus exclusively in carrier isolates and the attenuation of an *eqbE* deletion mutant in Welsh mountain ponies, confirms its importance to the natural disease. We identify loci that may similarly be required for the full virulence of *S. equi*, directing future research into the evolution of this host-restricted pathogen towards its eradication.

SU06
Regulatory phosphate-based molecules

SU06We0900

Magic Spot coordinates virulence of *Legionella*

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Legionella pneumophila is an opportunistic pathogen naturally found in fresh water supplies. When ingested by amoebae or protozoa, the bacteria avoid digestion and replicate inside the host cells. When humans inhale contaminated water droplets, *L. pneumophila* can also survive and replicate within lung macrophages. Since transmission of *L. pneumophila* from one person to another has never been reported, humans are a dead-end for this opportunistic pathogen. Instead, free-living protozoa and amoebae exert selective pressures for *L. pneumophila* to acquire strategies to promote survival and replication within professional phagocytes in the environment and the human lung.

To thrive in the environment *L. pneumophila* alternates between distinct cell types. A motile, infectious and resilient form is equipped for transmission from one host cell to another, whereas a more sensitive and non-motile cell type grows in vacuoles of amoebae and macrophages. This life cycle is controlled by metabolic cues. When nutrients are plentiful within the host cell, transmissive bacteria switch to the replicative form and grow to large numbers. Conversely, when amino acids and fatty acids become scarce in the host cell, the stringent response pathway equips *L. pneumophila* to stop replicating and express its panel of virulence traits to promote transmission.

SU06We0930

The role of ppGpp in modulating the expression of *Salmonella typhimurium* pathogenicity islands

Arthur Thompson

Salmonella *Molecular Microbiology Group, Gut Health & Food Safety, Institute of Food Research, Norwich Research Park, Norwich, UK*

The pathogenicity of *Salmonella* Typhimurium is, to a large extent, dependent on two horizontally acquired pathogenicity islands, SPI1 and SPI2. In addition to regulatory proteins, each cluster encodes a type III secretion system (T3SS) which forms a needle like complex on the bacterial surface and injects effector proteins into the cytosol of host cells. These effectors manipulate cellular functions of the host cell and thus facilitate the progression of infection. SPI1 is primarily required for *Salmonella* invasion of host epithelial cells whilst SPI2 is required for intracellular survival and replication within phagocytic cells. In this presentation the major role ppGpp plays in activating expression of SPI1, SPI2 and other *Salmonella* virulence-related genes in response to environmental stimuli will be described. How the ppGpp-dependent expression of SPI1 and SPI2 genes are also modulated by the RNAP accessory protein DksA and the stationary phase sigma factor, RpoS will be presented.

SU06We1000

Epigenetic control of bacterial persistence by (p)ppGpp

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Persistence refers to the phenomenon that isogenic populations of antibiotic-sensitive bacteria produce rare cells that transiently become multidrug tolerant. It has been proposed that the multidrug tolerance originates from slow growth of the rare cells. However, this has never been shown directly with any wild type bacterium. Here we show that an exponentially growing population of wild type *Escherichia coli* cells produce rare cells that stochastically switch into slow growth, that the slow-growing cells are multidrug tolerant and that they are able to resuscitate. The persistence phenotype depended hierarchically on (p)ppGpp, Lon protease, inorganic polyphosphate and toxin – antitoxins. We present evidence that the level of (p)ppGpp varies stochastically in a population of exponentially growing cells and that the high (p)ppGpp level in rare cells induces slow growth and persistence. (p)ppGpp triggers slow growth by activating toxin – antitoxin loci through a regulatory cascade depending on inorganic polyphosphate and Lon protease. (p)ppGpp was also required for persistence during stationary phase and during biofilm formation. Combined with previous results[1], these observations raise the possibility that (p)ppGpp and toxin – antitoxin genes mediate persistence in pathogenic bacteria.

(1) Maisonneuve *et al.* (2011). Bacterial persistence by RNA endonucleases. ***PNAS*** *108*, 13206–13211

SU06We1100

Offered paper **Understanding the stringent response in *Yersinia pestis***

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The rise in antibiotic resistance, combined with the paucity of novel antimicrobials, has become a matter of intense concern. Similarly, innate, emerging and even engineered resistance is of disquiet for pathogens of interest in biodefence. Therefore a significant need to identify novel classes of antibiotics exists. Under conditions of nutrient limitation, bacteria initiate the stringent response, co-ordinated by the signalling nucleotides guanosine tetra- and penta-phosphate, collectively termed (p)ppGpp. During starvation, (p)ppGpp accumulates and coordinates diverse transcriptional alterations. (p)ppGpp levels are controlled by two enzymes, RelA and SpoT.Inorganic polyphosphate, a global regulatory molecule, has also been linked to the stringent response. Levels of polyphosphate are controlled by a polyphosphate kinase enzyme, PpK and an exopolyphosphatase, PpX. Mutation of relA and spoT results not only in the abrogation of (p)ppGpp production, but also in lower levels of polyphosphate accumulation. However, the interaction of the stringent response with the polyphosphate regulon is not clearly understood.We present the identification of ppK and ppX genes in Yersinia pestis, their inactivation, and characterisation of these mutants in vitro and in vivo. This is compared to the effects of mutation of the stringent response genes.

SU06We1115

Polyphosphates in *Streptomyces*

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Polyphosphates (polyP) are energy and phosphate storage polymers playing key roles in the cellular metabolism of all living organisms. The dynamic of the polyP content in relation with the Pi concentration of the growth medium and the intracellular ATP and ADP concentration was assessed in the wild type, the *phoP* and *ppk* mutants of *Streptomyces lividans* TK24 as well as in *S. coelicolor* M145. Abundant polyP were shown present in the spores of all these strains. Experimental datas suggested that neither the regulator PhoP, nor the polyphosphate kinase Ppk were involved in PolyP biosynthesis. In contrast, both PhoP and Ppk were shown to play a positive role in PolyP degradation, in condition of Pi limitation. In these conditions, the intracellular concentration of ADP was consistently higher in the *ppk* mutant strain than in the *wt* strain suggesting that Ppk was acting as a Adenosine Di Phosphate Kinase, regenerating ATP from ADP and polyP. In the absence of Ppk, the bacteria would experience energetic stress and some homeostatic mechanisms would be triggered in order to re-establish its energetic balance. Indeed, at late stationary phase, the ATP content of the *ppk* mutant was shown to be higher than that of the *wt* strain.

SU06We1145

Offered paper ***Lactobacillus reuteri*, microcompartments, and polyphosphate metabolism**

KAREN McCARTHY[1], Alan Barry[1], Minzghi Liang[1], Steffi Frank[2], Martin J. Warren[2], Michael B. Prentice[1]

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Lactobacillus reuteri strains are probiotic GRAS (generally regarded as safe) organisms found in the enteric microbiotia of a wide variety of vertebrates including humans. Human and poultry-associated strains make microcompartments when induced by certain metabolites (1,2-propanediol and glycerol). These are 70-150 nm polyhedral structures containing enzymes enclosed by a thin pore-containing protein shell. *L. reuteri* also contains polyphosphate kinase (PPK1) which reversibly catalyses the polymerisation of the terminal phosphate of ATP into a polyphosphate chain. Polyphosphate polymer is frequently detected as volutin granules in Lactic acid bacteria, and has also been associated with the microcompartments of photosynthetic cyanobacteria. We present properties of recombinant *L. reuteri* PPK1 and discuss native and potential novel bioengineered

connections between polyphosphate metabolism and microcompartments in *L. reuteri*.

SU06We1300

How pathogens subvert cellular phosphate-based regulatory molecules to their own advantage: *Salmonella* Type III effectors in action

Amin Tahoun[1], Simmi Mahajan[1], Edith Paxton[1], David S. Donaldson[1], Darren J. Shaw[1], David L. Gally[1], Andreas Lengeling[1], Neil A. Mabbott[1], Jürgen Haas[2], ARVIND MAHAJAN[1]*

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S. Typhimurium targets antigen-sampling microfold (M) cells as the preferred cell type to translocate across the gut epithelium. Although M cells represent a small proportion of the specialised follicular associated epithelium (FAE) overlying mucosa-associated lymphoid tissues, their density increases during Salmonella infection. The molecular mechanism underlying this Salmonella-mediated increase in M cell density was uncertain. Using *in vitro* and *in vivo* infection models we demonstrate that the *S.* Typhimurium type III effector protein SopB induces an epithelial-mesenchymal transition (EMT) of FAE enterocytes into M cells. This cellular trans-differentiation depends on the activation of Wnt/b-catenin signalling leading to induction of both RANKL and its receptor RANK. The autocrine activation of RelB expressing FAE enterocytes by RANKL/RANK induces EMT regulator Slug that marks epithelial trans-differentiation into M cells. On going work has identified other complementary set of signalling molecules that can de-differentiates intestinal epithelial cells and thus facilitates successful colonisation of host. This study demonstrates a novel host-pathogen interaction in which *S.* Typhimurium transforms primed epithelial cells into M cells to promote host colonisation and invasion.

SU06We1330

Cyclic di-GMP-responsive riboswitches in the human pathogen *Clostridium difficile*

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Clostridium difficile-associated diarrhoea is currently the most frequently occurring nosocomial diarrhoea in Europe. Our recent deep sequencing data strongly suggest the importance of RNA-based mechanisms for the control of gene expression in this pathogen. More than 200 putative regulatory RNAs were identified including a variety of riboswitches. We demonstrated the functionality of a particular class of riboswitches responding to cyclic di-GMP, a universal bacterial signalling molecule controlling lifestyle switches from free-living motile state to biofilm communities and virulence in bacteria. In most microorganisms, proteins are effectors for c-di-GMP signalling pathways. In *C. difficile*, the RNA riboswitches are used as effectors sensing c-di-GMP. In contrast to most Gram-positive bacteria, *C. difficile* encodes a large number of c-di-GMP turnover enzymes. Moreover, 12 type I c-di-GMP-specific riboswitches and 4 type II c-di-GMP-dependent riboswitches have been predicted in *C. difficile*. Expression of all 16 predicted riboswitches was observed and experimental evidence for their regulatory role was obtained for coordinated control of many processes crucial for successful development of *C. difficile* inside the host such as adhesion, colonisation, biofilm formation, motility and other related processes. Altogether, our data further highlight the crucial role of this second messenger in this emergent enteropathogen.

SU06We1400

Offered paper **Phosphodiesterase signalling in the predatory bacterium *Bdellovibrio bacteriovorus***

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Bdellovibrio bacteriovorus is a predatory gram negative bacterium, which grows by entering in to the periplasm of other Gram negative species and uses the nutrients of the prey to grow. Growth within the prey periplasm is a process demanding large amounts of phosphate to replicate the nucleoid, expand the inner and outer membranes to produce several progeny per prey cell. Phosphate-containing compounds such as ATP are also required in the energetic transport of nutrients and enzymes between the prey periplasm and *Bdellovibrio*. Also cyclic di GMP is a phosphate-containing signal molecule essential to control predation (1).

We have been asking: What are the roles of phosphodiesterase activity during these processes? Two types of phosphodiesterase activity have been investigated. The first, exopolyphosphatases, break down long chain polyphosphates. Gene deletion has show that these are responsible for controlling the shape of the *Bdellovibrio* produced whilst dividing within a prey cell. A third exopolyphosphatase, and also a second type of phosphodiesterase, (with an HD-GYP domain which degrades the signalling molecule cyclic-di-GMP) participate in controlling the transition from the predatory lifestyle to a prey-independent lifestyle.

(1) Hobley *et al.* 2012 PloS Pathogens 8. (2)e1002493. doi: 10.1371/journal.ppat.1002493

SU06We1415

Offered paper **Characterisation of 'degenerate' cyclic di-GMP signalling proteins in *Escherichia coli***

NICOLA WHITING, Jeffrey Green

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Cyclic di-GMP is a recently discovered signalling pathway in *Bacteria* which ultimately controls the transition of bacteria between planktonic and biofilm lifestyles depending on environmental cues.

Two groups of enzyme make and break the secondary messenger c-di-GMP, each group containing a consensus motif of amino acid residues in the active site. However proteins exist which contain non-conserved motifs and are likely to be catalytically inactive or 'degenerate'. It is thought that these proteins fulfil a regulatory role.

This project focuses on two 'degenerate' cyclic di-GMP signalling proteins (YdiV and YeaI) in *E. coli* with the aim to understand what these individual proteins do as part of the wider signalling network.

Experiments confirm that both proteins are catalytically inactive and their abilities to bind potential ligands such as c-di-GMP and related molecules have been investigated.

Functional characterisation of YdiV, has shown that *ydiv* over-expression causes a decrease in motility by direct de-regulation of flagellar synthesis. The YdiV protein binds to a transcription factor $FlhD_4C_2$, and affects the DNA-binding and transcriptional activity of $FlhD_4C_2$.

SU06We1500

Cyclic-AMP and CRP proteins in mycobacteria

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Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), a major worldwide healthcare problem. *Mycobacterium tuberculosis* possesses multiple adenylate cyclase proteins that generate cyclic-AMP from ATP. It has long been known that mycobacteria secrete cyclic-AMP (cAMP) and it now recognised that cAMP intoxication of human alveolar macrophages is likely to be important in TB pathogenesis. In addition to modulating host signalling pathways, cAMP is perceived by the Cyclic-AMP Receptor Protein (CRP) to regulate bacterial gene expression. The *M. tuberculosis crp* mutant is attenuated and the interaction between CRP and cAMP differs from the *Escherichia coli* paradigm and the *M. smegmatis* CRP – cAMP binds two independent sites in the *M. tuberculosis* CRP dimer and cAMP-binding is not essential for CRP gene regulatory activity *in vitro* or *in vivo*. Nevertheless, *M. tuberculosis* CRP is a global regulator that acts as both classical transcription factor and possibly as a nucleoid associated protein. Amongst the genes controlled by CRP is *whiB1*, which encodes an essential, iron-sulfur protein that acts a nitric oxide-responsive transcription regulator. Thus, cAMP-signalling is closely linked to establishing an infection in macrophages and responses to host defence strategies.

SU06We1530

To stick or swim: cyclic di-GMP control of bacterial biofilm formation and motility

Christopher M. Waters

Department of Microbiology and Molecular Genetics, Michigan State University, USA

A major challenge faced by bacteria is to sense and adapt to an ever-changing environment. Cyclic di-GMP (c-di-GMP) is a newly appreciated second messenger that controls the switch between a motile and sessile lifestyle in response to different environmental cues. The environmental signals that impact c-di-GMP levels and the molecular mechanisms by which changes in the levels of c-di-GMP regulate downstream gene expression are poorly understood. We have determined that intestinal bile increases the levels of c-di-GMP in *Vibrio cholerae*. The response of *V. cholerae* to bile is pH dependent implicating this process as potentially important for intestinal colonisation. We are also exploring how c-di-GMP impacts gene expression both at the level of transcription induction and post-transcription gene control. I will discuss our analysis of two transcription factors in *V. cholerae* that are positively and negatively regulated by c-di-GMP, and our discovery of a new regulatory paradigm whereby binding of c-di-GMP to a riboswitch located at the 3'-end of a small RNA (sRNA) increases its stability.

SU06We1600

c-di-AMP: a novel second messenger in bacteria with diverse effects

Angelika Gründling

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Nucleotide signaling molecules control fundamental processes in all forms of life. In particular cyclic-dinucleotides have gained recently increased attention with the discovery of their production not only by bacterial cells but also eukaryotic cells and the identification of novel nucleotides such as c-di-AMP. c-di-AMP has now been shown to be synthesised by a range of Gram-positive bacteria, including important human pathogens such as *Staphylococcus aureus, Streptococcus pyogenes* and *Listeria monocytogenes*. There is now increasing experimental evidence that c-di-AMP controls essential cellular processes within bacterial cells. This notion is corroborated by our own findings that *S. aureus* strains defective in c-di-AMP have sever growth and morphological defects. Canonical bacterial signaling molecules act through specific receptor proteins by direct binding to alter their activity. To identify c-di-AMP receptor proteins and hence cellular pathways controlled by it, we used a combination of affinity pull down assays, bioinformatics analysis and a systematic whole genome protein/nucleotide interaction screen. Using these methods, we identification four *S. aureus* c-di-AMP receptor proteins that are also widely distributed among other bacteria. With the identification of these receptor proteins we directly link for the first time the c-di-AMP signaling network to a central process in bacteria, that is ion homeostasis.

SU07
Microbial survival in the host

SU07We0900

Connections between nutrient acquisition and virulence in *Cryptococcus neoformans*

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We seek to understand the mechanisms that allow *Cryptococcus neoformans* to proliferate in the vertebrate host environment and to cause meningoencephalitis in humans. To achieve this goal, we employed molecular genetic and genomic approaches to identify the *C. neoformans* factors for metabolic adaptation and for the elaboration of virulence during cryptococcosis. These processes are tightly integrated and this fact is illustrated by the connections between iron acquisition and expression of the major virulence determinant, the polysaccharide capsule. A key area of investigation focuses on identifying and characterising the mechanisms of iron acquisition during infection, including the use of transferrin via a high affinity uptake system and the use of heme by a hemophore/endocytosis pathway. In recent work, we employed an insertional mutagenesis approach to identify additional functions involved in heme utilisation including ferric reductases. This analysis reinforced the importance of endocytosis in iron acquisition and revealed a role in capsule production. Parallel work has focused on central carbon metabolism and the production/utilisation of acetyl-CoA via beta-oxidation and ATP citrate lyase, as well as the import of phosphate and production of polyphosphate. These processes contribute to virulence in *C. neoformans* and reveal a myriad of interconnections between nutritional adaptation and cryptococcosis.

SU07We0930

Antigenic variation in African trypanosomes: mechanisms of allelic exclusion

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Antigenic variation in African trypanosomes requires monotelomeric transcription of a single Variant Surface Glycoprotein (*VSG*) Expression Site (ES) and the concomitant silencing of other *VSG*s at ESs. This allelic exclusion is essential for host immune evasion and persistence, but the mechanisms underlying the process are poorly understood. We performed a genome-scale RNA interference library screen for loss of silencing in bloodstream form *T. brucei*. The screen identified VEX1 (for Vsg EXclusion), that is associated with an extranucleolar RNA polymerase I compartment known as the Expression Site Body. VEX1 knockdown lead to loss of *VSG* silencing, resulting in cells coated with multiple VSGs. In another study, we found a role for ES-derived transcripts in allelic exclusion. Telomere-mediated fragmentation was used to engineer reporter cassettes adjacent to *de novo* telomeres, revealing genetic interference among homologous sequences. We also demonstrated derepression of telomeric transcripts triggered by knockdown of other telomeric transcripts. Thus, sequence-specific genetic interference mediates crosstalk among telomeric ESs; we propose that RNA from the active site forms a diffusible silencing signal. In addition, VEX1 marks the active locus and is required to maintain allelic exclusion. Similar mechanisms could underlie other examples of allelic exclusion in parasites and beyond.

SU07We1000

Offered paper **A fatty solution to high salt in *Candida albicans*?**

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We have investigated the effect of micronutrient (iron, zinc, manganese and copper) limitation on *Candida albicans* physiology. Prolonged zinc starvation resulted in the formation of giant *C. albicans* yeast cells which were more efficiently cleared by human neutrophils. These "Goliath cells" accumulated large intracellular vesicles, which Nile Red staining indicated were composed of lipids.

Lipid droplets are known to contain high levels of triacylglycerol, which can be hydrolysed to fatty acids and glycerol. We hypothesised that lipid droplets may serve as a reservoir for glycerol production in response to environmental stresses.

Indeed, we observed rapid and sustained lipid droplet depletion in response to osmotic stress in *C. albicans*, suggesting that fatty acids and glycerol may be produced from lipid droplets in response to this environmental stimulus.

To investigate this further, we deleted a putative lipid droplet-associated acylglycerol lipase in *C. albicans*. The resultant mutant exhibited aberrant lipid droplet homeostasis, was not able to break down these vesicles in response to osmotic stimulus and could not grow in the presence of high salt levels.

Together these observations suggest a novel mechanism of osmo-protection in *C. albicans*.

SU07We1015

Offered paper **The homologues of the mammalian endocytic AP-2 adaptor complex μ subunit have a role in polarised growth in *Saccharomyces cerevisiae* and *Candida albicans***

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The μ subunit of the AP-2 adaptor complex of mammals is a known cargo binding protein required for the internalisation of some cargo molecules into the cell via clathrin mediated endocytosis. There is some evidence that indicates that the same is true in the yeast Saccharomyces cerevisiae. Our investigation into the possible role of the μ subunit as an endocytic cargo binding protein in yeast has led us to discover its involvement in the proper establishment and maintenance of cell polarity both during pseudohyphal growth and during mating projection formation in S. cerevisiae, as well as in the response to cell wall integrity damage. Interestingly, we have also discovered the involvement of the homologue of the AP-2 complex μ subunit in the regulation of cell polarity during filamentous and invasive growth in the fungal pathogen of humans Candida albicans. Our results, together with previous findings by others, suggest a possible regulatory link between the processes of endocytosis and polarised growth.

SU07We1100

The molecular basis of invasive growth in the pathogenic mould *Aspergillus fumigatus*

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In lung diseases caused by the mould *Aspergillus fumigatus* epithelial destruction is thought to be mediated by proteases of fungal origin. We have found that the *A. fumigatus* pH-responsive transcription factor PacC governs expression of secreted proteases during invasive lung infections and is required for epithelial invasion and pathogenicity. *In-* and *ex-vivo* analyses of infected epithelia revealed that *A. fumigatus* elicits a series of distinct, and sequentially implemented assaults upon epithelial integrity, consisting of (I) epithelial entry (II) cell wall-mediated epithelial disaggregation and (III) protease-mediated damage, all of which are deficient in the Δ*pacC* mutant. Though damaging, internalisation of *A. fumigatus* spores by alveolar epithelial cells was found, via nystatin protection assays, to promote fungal clearance. Concordantly, spores of the Δ*pacC* mutant persisted in the lungs of leukopenic mice. Aberrant remodelling of the Δ*pacC* cell wall during initiation of mammalian infection suggested the β-glucan receptor, Dectin-1, as mediating spore internalisation, a hypothesis which was proven by the anti-internaisation activity of an anti-Dectin 1 antibody *in vitro*. Consistent with a curative role for spore internalisation, pulmonary damage was heightened in leukopenic Dectin-1$^{-/-}$ mice relative to wild type counterparts. Our findings shift the pathophysiological profile of this everyday host-pathogen interaction to one in which neutrophil titre presents a risk which is secondary to competence of epithelial defences, and sets a new precedent for the unified study of the diverse pulmonary complications caused by *A. fumigatus*.

SU07We1130

Life without promoters: genomic, transcriptomic and proteomic mechanisms of environmental adaptation in the eukaryotic pathogen *Leishmania donovani*

Pablo Prieto Barja[1], Pascale Pescher[2], Fatma Guerfali[3], Darek Kedra[1], Robin Friedman[4], Mathieu Cayla[2], Benno Schwikowski[4], Cedric Notredame[1], GERALD F. SPÄTH[2]

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Protozoan pathogens of the genus *Leishmania* cause severe diseases in humans, termed leishmaniases, which rely on the parasite's capacity to infect and thrive inside phagocytic immune cells, including tissue macrophages. A major hallmark

of *Leishmania* biology is represented by its remarkable ability to adapt to changing environments despite the largely constitutive expression of its genetic material and the absence of classical transcriptional regulation. The underlying mechanisms are largely unknown even though they may play important roles in parasite adaptation to new animal reservoirs, host tissues or drug pressure. This presentation will focus on a systems-level comparative analysis of proteomic, transcriptomic, and genomic parasite responses *in vivo* in infected hamsters and *in vitro* after adaptation to axenic culture. Quantitative proteome profiling by 2D-DiGE analysis revealed important phenotypic traits in hamster-derived parasites that are under selection by leishmanicidal host activities and that adapt *L. donovani* for intracellular proliferation. Deep sequencing of both genome and transcriptome uncovered important alterations of axenic parasites, including multiple chromosomal deletions covering for example a folate/biopterin (FT/BT) transporter previously linked to parasite infectivity. Furthermore, axenic parasites show amplification of the chromosomes 1, 5, 23, and 26, likely as a result of purifying and positive selection towards fast *in vitro* proliferation. Significantly, these amplifications only partially correlate with increased transcript abundance suggesting that dosage compensation is operational in *Leishmania*. The role of post-transcriptional and epigenetic regulation of transcript abundance will be discussed.

SU07We1300

Offered paper **Adaptive response of extra-intestinal pathogenic *Escherichia coli* to human serum**

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Surveillance programs have identified *Escherichia coli* as the most frequently isolated Gram-negative organism causing bloodstream infections (BSI). BSI are predominantly caused by extra-intestinal *E. coli* (ExPEC). The presence of *E. coli* in the blood stream can lead to the development of sepsis syndrome, severe sepsis or septic shock.

ExPEC strains can resist the killing activity of human serum through expression of virulence factors allowing this organism to survive in the blood stream and establish bacteraemia. In this study gene expression of ExPEC strain CFT073 was analyzed in the presence of human serum and heat inactivated serum. Exposure to active serum resulted in upregulation of genes involved in stress response, regulating cell envelope synthesis, exopolysaccharide production and genes encoding lysozyme inhibitors.

The genes most highly upregulated by active serum were found to belong to the Rcs regulon. The Rcs two component system controls expression of over 150 genes involved in cell envelope function, including the upregulated exopolysaccaride genes and lysozyme inhibitors identified in this study. These factors were investigated to determine whether they contribute to the ability of extra-intestinal *E. coli* to survive in serum.

SU07We1315

Offered paper **DNA checkpoint kinases as peroxide sensors and regulators of filamentous growth in *Candida albicans***

Alessandra da Silva Dantas[1], Adam Crawshaw[1], Brian Morgan[1], Lars-Peter Erwig[2], JANET QUINN[1]

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The ability of *Candida albicans* to sense and respond to H_2O_2, generated by host innate defences, is essential for virulence. We have recently shown that H_2O_2 induces filamentous growth in *C. albicans* via activation of the DNA checkpoint kinase Rad53. In addition, we found that the oxidoreductase thioredoxin protein Trx1 negatively regulates H_2O_2-induced Rad53 activation, with *trx1*Δ cells forming filaments in the absence of H_2O_2 due to constitutive activation of Rad53. Here we have defined the pathway mediating H_2O_2-induced activation of Rad53, and explored the mechanism underlying Trx1 regulation of Rad53. The upstream sensing kinase Mec1^{ATR} and the Rad9 adaptor protein are essential for Rad53 activation in response to H_2O_2-mediated DNA damage. Significantly, however, the Tel1^{ATM} sensing kinase regulates Rad53-dependent filamentous growth in response to H_2O_2 independently of activation of the DNA damage response. Instead, Tel1^{ATM} is oxidised in response to H_2O_2 which results in Rad53 activation and filamentous growth. We illustrate that Trx1 functions to reduce Tel1^{ATM}, and that the filamentous phenotype of *trx1*Δ cells is due to the accumulation of oxidised Tel1^{ATM}. We also show that activation of Rad53 via Mec1^{ATR}, but not Tel1^{ATM}, is vital for *C. albicans* to survive the hostile environment of the macrophage phagosome.

SU07We1330

The adaptation of *Candida albicans* to complex host niches

Alistair J.P. Brown

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Candida albicans is capable of thriving within diverse host niches. This fungus is a commensal of the skin, gastrointestinal and urogenital tracts, it causes mucosal infections (thrush), and it causes life-threatening systemic infections in immunocompromised patients. It has become clear that fitness attributes (as well as virulence factors) are critical for the pathogenicity of *C. albicans*. For example, nutrient adaptation as well as oxidative, nitrosative, osmotic and thermal stress responses promote the virulence of this fungus. Numerous groups are contributing to the elaboration of these adaptive responses and of the regulatory networks that control them. We have found that these adaptive responses are intimately linked. For example, certain combinations of stress trigger unexpected and non-additive responses in *C. albicans* cells, and our data suggest that these combinatorial stresses contribute to the potency of host phagocytes in clearing fungal infections. Also, the resistance of *C. albicans* to osmotic and oxidative stresses is dramatically affected by local nutrients. Furthermore, changes in carbon source affect antifungal drug resistance, immune surveillance and virulence. The nature of these combinatorial adaptive responses and their relevance to the survival of *C. albicans* within the host will be discussed.

SU07We1400

Offered paper **Combinatorial stress response in the fungal pathogen *Candida glabrata***

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Candida glabrata is an opportunistic pathogen with an increasing incidence and resistance to antifungal drug treatment. Previous studies have only focused on the response to independent stressors, therefore little is known about the adaptive response to simultaneous stresses, even though this is likely to be more relevant in an ecological and pathophysiological setting (i.e upon macrophage engulfment).

Timecourse microarray experiments conducted under defined doses of hyperosmotic (sodium chloride) and oxidative (hydrogen peroxide) stress, singly and in combination have identified differentially regulated transcripts unique to

simultaneous stress and not seen under single stress conditions. This supports our hypothesis that a specific transcriptional response is required for *C. glabrata* to respond and adapt to simultaneous stresses and cannot be fully explained by simply combining the transcript profiles of each single stress. Phenotypic screening of a *C. glabrata* null mutant library under single and combinatorial stress and the utilisation of *ex vivo* models of infection have been used to investigate this further.

Analysis of these transcript profiles show similarities with published *C. glabrata ex vivo* datasets, with the regulation of processes important in host survival showing that combinatorial stress *in vitro* can give biological insights into the host environment.

SU07We1415

Offered paper **A zebrafish model of cryptococcosis for the study of host–pathogen interactions *in vivo***

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Cryptococcus neoformans is a significant fungal pathogen of the immunocompromised, especially AIDS patients. A major barrier in the understanding of these infections has been an inability to put the molecular and cellular data we have on pathogenesis into the context of the initiation and progression of disease. Both aspects can be studied in the zebrafish, offering the opportunity to integrate molecular cell biology with a vertebrate model of infection, in particular how the outcome of the interaction between macrophages and *Cryptococcus* determines the outcome of disease.

Infection of zebrafish with *Cryptococcus* results in control and clearance of the pathogen at low doses but can lead to fungemia where immune cells are overwhelmed. Infection rarely disseminates from the blood stream in the immune competent zebrafish. Combining this infection model with sub-cellular level imaging throughout the zebrafish and genetically encoded and exogenous fluorescent markers and sensors we are able to dissect the course of infection and the subsequent host responses. Thus, with our model we can a) Study in vivo the interactions of macrophages and cryptococci in the context of disease progression b) Quantitate measurements of disease parameters and c) Undertake multi-variate analysis of interactions in determinants of disease progression.

SU07We1430

Trying to understand antigenic variation in malaria

Thomas Otto[1], Sammy Assefa[1], Mandy Saunders[1], Jacob Lemieux[2], Zoe Christodolou[2], Sue Kyes[2], Bob Pinches[2], Dominic Kwiatkowski[1], Matt Berriman[1], CHRIS NEWBOLD[1,2]

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Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) are a family of proteins that are expressed on the surface of *P. falciparum* infected red cells (irbc). These proteins are important virulence factors because they mediate infected red cell binding to a variety of host cell types and concentrate irbc in organs leading to organ dysfunction. They are also important targets of the host protective antibody response. These proteins are encoded by the highly polymorphic var multi-gene family and avoid the antibody response by successive transcriptional switches between family members through a process of antigenic variation. This gene and corresponding protein family are thus both central to both pathogenesis and immunity. We have recently obtained data on the overall kinetics of the transcriptional switching pathway and on the accompanying changes that occur in genome wide DNA organisation. We have also managed to assemble complete *var* gene repertoires from ~1500 field isolates resulting on sequence data on ~100,000 genes and shown an unexpected level of sequence sharing across isolates and across continents. The implications of these data for the understanding of the role of var genes in malaria will be discussed.

SU07We1530

***Candida* survival strategies in the host**

Bernhard Hube

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Candida albicans and *C. glabrata* are both harmless commensals of mucosal surfaces in healthy individuals and aggressive pathogenic yeasts in susceptible hosts. In the commensal phase, the fungi attach to host surfaces, co-exist and interact with the bacterial microflora, aquire nutrients accessible on mucosal surfaces, adapt to local conditions such as varying pH values and oxygen levels and replicate without causing inflammation. These conditions change dramatically during the transition to a pathogenic growth style. This transition includes invasion into host tissues and damage of epithelial cells and requires nutrient acquisition, including metals like iron and zinc, directly from host cells and molecules. Uncontrolled proliferation, invasion and damage in turn cause inflammation and recruitment of phagocytic cells. To counteract the killing mechanisms of phagocytes, the two "unlike cousins", *C. albicans* and *C. glabrata* (Brunke and Hube (2013), *Cell Microbiol* 15:701-8) have developed distinct survival strategies, including detoxification of toxic compounds, intracellular survival and replication and escape mechanisms.

SU07We1600

Genotypic diversity, a survival strategy for the apicomplexan parasite *Theileria parva*

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Tick-borne *Theileria parva* causes a severe lymphoproliferative disease in cattle in sub-Saharan Africa. The parasite specific protective cytotoxic T lymphocyte response is tightly focused on a limited number of polymorphic epitopes. In individual animals only one or two antigens are recognised. Little is known about how *T. parva* genetic diversity was generated or its function. A genome wide satellite marker set was used to characterise existing *T. parva* parasite stabilates to determine how cattle-tick passages affected their composition. Stabilate 72 (St72), was passaged 5x since isolation from the field and is dominated by a single genotype, 72-01. No switching of predominant genotypes was observed when St72 was passaged through naïve animals. However, this resulted in further inbreeding of the 72-01 genotype, decreasing allelic variation but revealing extensive recombination. Passage of St72 through animals, immunised against the 72-01 genotype, resulted in the elimination of this genotype during early infection, while other genotypes managed to establish. These results suggest that extensive recombination allows the generation of new parasite genotypes and increase genetic diversity at a population level. Some of these genotypes, when infecting immune cattle are likely to evade the tightly

focused immune response and thus result in survival of the parasite.

SU07We1630

Understanding intracellular parasitism by fungi

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My group are interested in host-pathogen interactions and, in particular, in understanding how some pathogens are able to subvert the innate immune system. Most of our work focuses on phagocytic cells, which some microorganisms are able to use as a 'safe house' within which to replicate.

In this talk, I will focus on cryptococcosis, a leading and frequently fatal fungal infection. A key feature of this disease is that the infectious agent is not efficiently killed by phagocytic cells of the innate immune system. We therefore study the events that lead both to phagosomal persistence of this organism and, in some cases, to a novel escape process termed 'vomocytosis'. We are also interested in the genetic changes that drive hypervirulent outbreaks of cryptococcosis and what the cellular consequences are of such changes. Finally, I will compare this situation with the intracellular behavior of the rare but highly aggressive fungal disease zygomycosis, which is often a fatal complication in patients with major trauma wounds.

SU07We1700

By-pass of human serum killing by African trypanosomes

Paul Capewell[1§], Caroline Clucas[1§], Eric DeJesus[2], Rudo Kieft[2], Stephen Hajduk[2] Nicola Veitch[1], Pieter C. Steketee[1], Anneli Cooper[1], William Weir[1], ANNETTE MACLEOD[1]

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Trypanosoma brucei gambiense causes 97% of all cases of African sleeping sickness, a fatal disease of sub-Saharan Africa. Most species of trypanosome, such as *T. b. brucei*, are unable to infect humans due to the trypanolytic serum protein apolipoprotein-L1 (APOL1) delivered via two trypanosome lytic factors (TLF-1 and TLF-2). Understanding how *T. b. gambiense* overcomes these factors and infects humans is of major importance in the fight against this disease. Previous work indicated that a failure to take up TLF-1 in *T. b. gambiense* contributes to resistance to TLF-1, although another mechanism is required to overcome TLF-2. Here, we have identified a gene in *T. b. gambiense* that is essential for resistance to lysis. Deletion of the gene in *T. b. gambiense* renders the parasites sensitive to human serum and recombinant APOL1. Deletion of it in *T. b. gambiense* modified to uptake TLF-1 showed sensitivity to TLF-1, APOL1 and human serum. Reintroducing the gene into knockout parasite lines restored resistance. We conclude that it is essential for resistance in *T. b. gambiense*.

Posters

SU01
Microbial modulation of cellular pathways

SU01/01

Immunomodulatory effects of *Helicobacter pylori* membrane vesicles

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Helicobacter pylori infection of the human gastric mucosa persists lifelong, inducing chronic inflammation, which is associated with peptic ulceration and gastric adenocarcinoma. Bacterial membrane vesicles (MV) could potentially mediate delivery of immunomodulatory molecules to host immune cells, promoting persistence. However, bacterial MV effects on human immune cells remain largely uncharacterised to date.

We used MV carrying different forms of the vacuolating cytotoxin, VacA, to characterise the effects of *H. pylori* MV on human immune cells. The more toxigenic 60190 strain directed a greater proportion of its toxin (s1/i1 VacA) into vesicles than the SS1 strain (s2/i2 VacA), but engineering the SS1 strain to produce s1/i1 VacA did not increase the toxin content of its vesicles. Vesicles from all strains tested, including a 60190 isogenic mutant null for *vacA*, strongly induced IL-10 and IL-6 production by human peripheral blood mononuclear cells ($P < 0.001$) independent of the infection status of the donor. *H. pylori* MV induced T cell apoptosis and this was enhanced by ($P < 0.001$), but not completely dependent on, the carriage of VacA.

These findings suggest a role for *H. pylori* MV in stimulation of innate pro- and anti-inflammatory responses and in suppression of T cell immunity.

SU01/02

The role of the Tfs4 type IV secretion system in chromosomal DNA transfer in *Helicobacter pylori*

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Helicobacter pylori are a genetically diverse species due to polymorphism and intra- and intergenomic recombination promoting the loss and gain of chromosomal genes within the population. However, the mechanisms underlying gene acquisition are not clearly defined. Whereas the Com type IV secretion system (T4SS) is known to contribute to both DNA uptake and plasmid release, Com-independent mobilisation of chromosomal plasticity zone (PZ) *tfs4* gene clusters has also been reported.

In this study, we aim to define the role of the Tfs4 T4SS and key accessory proteins in the transfer of PZ and non-PZ genes.

Initial DNase I resistant mating assays were performed using mutant *H. pylori* strains inactivated in key components of each T4SS. Genes were disrupted by insertion of antibiotic resistance cassettes. Donor strains were incubated in the presence of DNase I and mated with isogenic spectinomycin resistant recipients. DNA transfer was demonstrated by selection for transconjugants on antibiotic plates.

Preliminary studies show that chromosomal DNA transfer is reduced in *tfs4* mutants with transfer efficiencies appearing to be strain dependent, perhaps due to non-reciprocal T4SS activity in different recipient strain backgrounds. Ongoing work will better distinguish between general and specific Tfs4 DNA transfer mechanisms and the substrates involved.

SU01/03

The putative lipoprotein VacJ contributes to serum resistance in *Burkholderia pseudomallei* and *Burkholderia thailandensis*

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Burkholderia pseudomallei is the causative agent of melioidosis. The gene *bpsl3147*, which encodes the putative lipoprotein VacJ, was identified in a screen for *B. pseudomallei* K96243 mutants that are attenuated in a mouse infection model and has been confirmed to be essential for full *in vivo* virulence. A highly conserved homologue of *vacJ* is present in the relatively avirulent *Burkholderia thailandensis.*

In this work, we studied the virulence factor VacJ using *B. thailandensis* as a comparative model. The *B. thailandensis* Δ*vacJ* mutant was susceptible to serum-mediated killing, suggesting that VacJ is important in preventing membrane attack complex formation from disrupting the outer membrane. Although the capsule (CPS) and lipopolysaccharide of *B. pseudomallei* were previously shown to contribute to survival in serum, VacJ appears to be an additional virulence factor that contributes to this role, as evidenced by the serum susceptibility of an acapsular *B. pseudomallei* Δ*vacJ* mutant. Of interest, a CPS I positive *B. pseudomallei* Δ*vacJ* mutant did not show this serum susceptibility in our *in vitro* assays.

Continued studies to characterise VacJ will help shed light on *B. pseudomallei* pathogenesis and explain the distinction in virulence phenotypes observed when comparing animal infection models and serum survival *in vitro* assays.

SU01/04

Host–pathogen interactions during biomaterial associated infection and the immunological deficit

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Bacterial infection is a major cause of medical device failure and associated morbidity. The presence of a foreign body such as an implant has been shown to greatly increase infection risk due to the creation of a local "immunological deficit". This "immunological deficit" is dependent upon the material implanted, the location of the implant and the patient. However, in general the presence of foreign material increases the ability of bacteria to evade immune responses, persist and infect surrounding tissue. Therefore, it is of importance to study how the presence of biomaterial associated infection influences the host immune responses and healing. To achieve this, various aspects of the immune responses to both the material alone and surfaces contaminated with clinically relevant bacteria have been studied to identify the combined effect of these factors. In addition, the ability of cells involved in healing, such as osteoblasts, fibroblasts and stem cells to adhere to biomaterials exposed to bacteria and bacterial components such as lipopolysaccharide, lipoteichoic acid and eDNA have also been measured. The results of these studies will be presented and the link between the host, pathogen and foreign body will be discussed.

SU01/05

***Salmonella* transforms follicle-associated epithelial cells into M cells to promote intestinal invasion**

AMIN TAHOUN

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S. Typhimurium targets antigen-sampling microfold (M) cells as the preferred cell type to translocate across the gut epithelium. Although M cells represent a small proportion of the specialised follicular associated epithelium (FAE) overlying mucosa-associated lymphoid tissues, their density increases during Salmonella infection. The molecular mechanism underlying this Salmonella-mediated increase in M cell density was uncertain. Using *in vitro* and *in vivo* infection models we demonstrate that the *S.* Typhimurium type III effector protein SopB induces an epithelial-mesenchymal transition (EMT) of FAE enterocytes into M cells. This cellular trans-differentiation depends on the activation of Wnt/b-catenin signalling leading to induction of both RANKL and its receptor RANK. The autocrine activation of RelB expressing FAE enterocytes by RANKL/RANK induces EMT regulator Slug that marks epithelial trans-differentiation into M cells. This study demonstrates a novel host-pathogen interaction in which *S.* Typhimurium transforms primed epithelial cells into M cells to promote host colonisation and invasion.

SU01/06

The search for a binding partner of a unique homologue of ubiquitin, produced by the commensal gastro-intestinal bacterium *Bacteroides fragilis*

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Bacteroides fragilis is an anaerobic, Gram negative commensal of the human gastro-intestinal tract. Recently, *B. fragilis* was found to encode and express a homologue of eukaryotic ubiquitin, BfUbb, which is known to be delivered to the periplasm and packaged into outer membrane vesicles. These vesicles are believed to transport proteins through the mucosa and deliver them to host epithelial cells via an unknown mechanism. There are currently no known binding partners for BfUbb in the host cell, however, likely targets include enzymes involved in the ubiquitination pathway. Due to key differences between the C-terminus of BfUbb and eukaryotic ubiquitin, BfUbb is able to terminate polyubiquitination *in vitro*, and may covalently bind E2 conjugating enzymes in the host cells. Such activity has the potential to interfere with a wide range of host cell processes, for example, inhibition of the inflammatory response. In this study we use surface plasmon resonance to determine the binding affinities of E2 enzymes for BfUbb and identify any E2 enzyme that binds covalently. This will provide some indication of the host cell pathway or pathways that *B. fragilis* is able to manipulate via this unique bacterial protein.

SU01/07

Do bacteria have a role in anti-cancer therapies?

Chris Merrett

University of Leeds, Leeds, UK

Selective targeting of tumours is a major hurdle in developing effective anti-cancer treatments. Bacteria offer a unique method of delivering therapeutics directly to the tumour, due to their innate predilection for colonising tumours, and here the extent of understanding and clinical application of this phenomenon is summarised and discussed. The tumour microenvironment exhibits many characteristics that make it ideal for bacterial entry and survival in the host. A wide range of therapies have been investigated which utilise bacteria as the vector, including gene directed enzyme prodrug therapy, immunotherapy, RNA interference and DNA vaccination. Many bacterial species are capable of directly lysing tumours. Non-invasive species can be engineered to express therapeutic proteins in the tumour environment, such as pro-drug converting enzymes, whereas invasive species are able to deliver genes or therapeutic agents directly into tumour cells. Combination therapies appear to be the most promising therapeutic avenue. Animal studies have been encouraging, and the relative safety of the treatment has been demonstrated. However, evidence of colonisation of human tumours or significant therapeutic effects in cancer patients is limited, and much work is still required before a successful therapy can be developed. Possible future directions of investigation are also outlined.

SU01/08

Epithelial mechanisms in the microbial pathogenesis of periodontal disease

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Periodontitis, a major cause of tooth loss, is a bacterially induced inflammatory disease that has been associated with certain bacterial species. Our aim is to identify the epithelial mechanisms activated by commensal species and periodontal pathogens to determine which signalling pathways, transcription factors and pro-inflammatory cytokines are associated with periodontitis. The H400 gingival epithelial cell line was infected with the health-associated Actinomyces naeslundii and periodontopathogens Fusobacterium nucleatum and Porphyromonas gingivalis for up to 2 hours for signal pathway activation or 24 h for cell damage and cytokine release. Whilst induction of p38 phosphorylation occurs by 5 min post-infection for all three bacteria, c-Fos appears after 1 h for A. naeslundii and P. gingivalis and 2 h for F. nucleatum. Induction of IL-1α and G-CSF was driven by all three bacteria, while IL-6 was only induced by A. naeslundii and F. nucleatum. IL-1β secretion was induced only by A. naeslundii, and GM-CSF secretion only by F. nucleatum. Bacterial-induced cell damage was not observed. Our data shows similarities in the epithelial response to these bacteria but also some key differences, which will form the basis of our future studies.

SU01/09

A unique homologue of eukaryotic ubiquitin produced by *Bacteroides fragilis*

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In the complete genome sequences of *B. fragilis* NCTC9343 and 638R, we discovered a gene, *ubb,* the product of which (BfUbb) has 63% identity to human ubiquitin. The sequence of *ubb* is closest in identity (76%) to the ubiquitin gene from a Migratory Grasshopper entomopoxvirus, suggesting acquisition by inter-kingdom horizontal gene transfer. A protein with such significant similarity to eukaryotic ubiquitin has not been discovered previously in prokaryotes. *ubb* is contained within a variable, low GC content region. Whole genome sequencing of a further six *B. fragilis* clinical isolates has been completed and here we present the comparison of the ubiquitin region of these strains. BfUbb has a predicted signal sequence; a processed form is detectable in whole cell extracts and in outer membrane vesicles (OMV). OMV may act as a delivery system for ubiquitin, providing a mechanism by which *B. fragilis* interacts with the host. These data indicate that the gastrointestinal tract of some individuals could contain a significant amount of aberrant ubiquitin with the potential to interfere with eukaryotic ubiquitin activity.

SU01/10

Identifying an O-antigen ligase in the human commensal *Bacteroides fragilis*

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Bacteroides fragilis is a Gram negative, obligate anaerobe and member of the normal mammalian gastrointestinal tract microbiota. *B. fragilis* is also an opportunistic pathogen and can cause, for example, peritonitis, soft tissue abscesses and bacteraemia. Capsular polysaccharides are virulence determinates for many pathogenic bacteria, however, the role(s) of *B. fragilis* polysaccharides in health and disease is complex. *B. fragilis* produces three phase-variable capsules: the large capsule (LC); small capsule (SC); and micro-capsule (MC). Within and between strain antigenic variability of *B. fragilis* MC is unprecedented. For example, three fully sequenced strains collectively have the potential to produce 28 different polysaccharides.

We previously hypothesised that the MC is linked to lipid A in a similar fashion to the O-antigen of enteric lipopolysaccharide (LPS); although the *B. fragilis* polysaccharide is of significantly higher molecular mass. WaaL in *E. coli* ligates the O-antigen to Lipid A-core during synthesis of LPS. Using bioinformatic analyses of WaaL homologues to ascertain commonalties in protein sequence or structure, we identified three potential genes encoding putative WaaL O-antigen glycosyltransferases in *B. fragilis* NCTC9343. Phenotypic analysis of markerless deletions of these genes, using immune-fluorescence microscopy and polysaccharide Immunoblotting, will be presented.

SU01/11

Interactions between the opportunistic pathogen *Bacteroides fragilis* and host proteins

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Bacteroides fragilis is a bacterium that resides in the human gastro-intestinal tract and is the most commonly isolated Gram-negative obligate anaerobe from human clinical infections, such as intra-abdominal sepsis, soft tissue abscesses and bacteraemia. The 54kDa *B. fragilis* fibrinogen binding protein (BF-FBP) binds human fibrinogen, the major structural component involved in fibrin-mediated abscess formation. The ability to bind and degrade fibrinogen may enhance bacterial escape from abscesses. *B. fragilis* clinical isolates can degrade fibrinogen via extracellular proteases. Here, four putative periplasmic protease encoding genes were deleted from *B. fragilis* NCTC 9343 to assess their functional role in the degradation of human fibrinogen. The ability of these mutants to inhibit gliadin hydrolysis, implicated in Celiac's disease, is also investigated. Complement activation is a major host extracellular defence against bacterial infection. Some pathogens inhibit complement activation by binding fibrinogen. The role of BF-FBP in relation to complement inactivation is being investigated by generation of deletion mutants and molecular characterisation of protein binding. These findings provide insight into the pathogenicity determinants of this important bacterium.

SU01/12

The role of Shiga-like toxin 2 in Enterohaemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 colonisation of intestinal epithelial cells

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Enterohaemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 possess the phage-encoded Shiga-like toxin (Stx) associated with haemorrhagic colitis and acute renal failure in humans. Cattle serve as the reservoir host remain unaffected by the colonisation, primarily occurring at the terminal rectum. The aim of this study is to identify the role of Stx in EHEC O157:H7 colonisation at the bovine terminal rectum. Ongoing work from cattle colonised with EHEC O157 has indicated a local cellular response including increased expression of Interferon-γ from cells from rectal lymph nodes following stimulation of cells with culture supernatants. Cell lines of different Gb3 (Stx receptor) expression and therefore different susceptibility levels towards Stx were then used to study EHEC O157-Stx-intestinal epithelium interactions. Predominately, the impacts of Interferon-γ and Stx on the cell cycle and inflammatory signalling were analysed by flow cytometry and immunoblotting.

SU01/13

Investigation of the impact of *Pseudomonas aeruginosa* alkyl-quinolone quorum-sensing (PQS) on initial innate immune recognition by human bronchia-epithelial cells

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Pseudomonas aeruginosa (Pa) is an opportunistic pathogen that attacks immune-compromised individuals especially patients suffering from cystic fibrosis (CF). *Pa* exploits a coordinated strategy called quorum-sensing (QS) to regulate gene expression and the 2-heptyl-3-hydroxy-4(1H)-quinolone, known as Pseudomonas quinolone signal (PQS), constitutes one of the QS systems. The involvement of PQS in *Pa* pathogenesis is supported by previous findings showing that PQS deficiency reduced *Pa* virulence in a mouse burn wound model and the wide distribution of PQS in the mucosal layer in CF patients. In this study, an *in vitro* infection model was established to investigate the impact of PQS on the innate recognition by airway epithelium. This was achieved by infecting human differentiated bronchial-epithelial cells (Calu-3) with PAO1 Lausanne (Wt) and its isogenic PQS-deficient mutant (Δ*pqsA-L*). Unexpectedly, although Δ*pqsA-L* is PQS deficient and lack of the virulence factor pyocyanin, no differences between Wt and Δ*pqsA-L* were detected regarding bacterial growth, cellular invasion, the amount of bacteria across the epithelial barrier, cytotoxicity or induction of proinflammatory cytokines. Both strains induce transcripts for GM-CSF, TNF-α, IL-6, IL-8 and IL-17c, largely remained cell-associated. Taken together, our findings indicated the *Pa* alkyl-quinolone QS is not required for initial innate immune recognition in the lung.

SU02
Fungal diseases, diagnostics and drug discovery (joint with BSMM)

SU02/01

Ascorbic acid abrogates susceptibility of *Candida albicans* to Hsp90 inhibitor geldanamycin in a Upc2-dependent manner

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Please note: Abstracts are published as received from the authors and are not subject to editing.

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The morphogenetic transitions of the opportunistic commensal Candida albicans are being influenced by temperature changes. It was already established that compromise of Hsp90 by elevated temperature, genetic depletion or pharmacological inhibition induces filamentation. In the present study, we show that several antioxidants such as cysteine, glutathione, dithiothreitol and ascorbic acid (Vitamin C) abolish the morphological shift induced by the Hsp90 inhibitor, geldanamycin (Gda). We focused on the non-thiol-containing antioxidant ascorbic acid and provide evidence that this ascorbic acid induced blockage is severely reduced in absence of the transcriptional regulator Upc2 and its target protein Erg11. We demonstrate that intracellular ergosterol levels are being decreased in the presence of Gda, while this decrease is being restored when co-applying ascorbic acid. In addition, we illustrate that Upc2 is also required for the antagonistic effect of ascorbic acid on inhibition of growth by fluconazole. These results identify Upc2 as the necessary transcriptional regulator for ascorbic acid induced effects and suggest an association between intracellular ergosterol abundance and Gda-induced elongated growth.

SU02/02

A comparison of three amphotericin formulations against biofilms of pathogenic *Candida* species under aerobic and anaerobic conditions

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Background Planktonic yeast cells cultured anaerobically can exhibit an increased MIC to Amphotericin. Little research has been done on the effectiveness of Amphotericin or its different formulations against biofilms of yeasts under similar conditions. Fungizone, Ambisome and Abelcet were therefore tested against biofilms of 48 *Candida sp.* isolates obtained from active clinical infections, under strict aerobic and anaerobic conditions.

Methods 8 each of *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. paraspilosis*, and 4 each of *C. kefyr*, *C. krusei*, *C. lusitaniae* and *C. guilliermondii* were cultured aerobically or anaerobically using an established 96 well microtitre plate method, with some modifications. 48hr old biofilms were exposed to concentrations of 1µg/ml, 3µg/ml, 10µg/ml and 30µg/ml of each drug for 24hrs. Reduction in metabolic activity was measured using XTT and compared to untreated controls.

Results Fungizone was most effective at reducing mean metabolic activity of all isolates (49%:30µg/ml). Ambisome was next effective, albeit at lower concentration (30%:3µg/ml). Abelcet was least effective (18%:30µg/ml). Little statistically significant variation in activity was noted between the two atmospheric conditions for any drug.

Conclusions This study shows that although Amphotericin remains the drug of choice for treatment of clinical biofilm infections of *Candida sp.*, consideration should be given to different formulations when determining treatment protocols.

SU02/03

Role of hypha formation and Ece1p in the commensal-pathogenic switch of *Candida albicans*

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The commensal/pathogenic switch of an opportunistic microbe is important for understanding its pathogenicity and its interactions with the host. We previously showed that epithelial cells (ECs) discriminate between yeast and hyphal forms of *Candida albicans* via a biphasic MAPK response, constituting MKP1 and c-Fos activation. We have now identified the hyphal-specific protein, Ece1p, as the fungal target recognised by ECs. We found that a *ece1*Δ/Δ mutant was unable to induce MKP1 phosphorylation or c-Fos production and was incapable of inducing IL-1a, IL-6, G-CSF or GM-CSF production or EC damage. Re-complementing one copy of *ECE1* restored cytokine production and the induction of p-MKP1 and c-Fos. Importantly, the inability of the *ece1*Δ/Δ mutant to activate ECs was not due deficiencies in hypha formation, adherence or invasion. Furthermore, a clinical commensal *C. albicans* isolate, 529L, which retains its ability to form hyphae but expresses comparatively low levels of *ECE1*, fails to induce MKP1 and c-Fos activation, damage and cytokines. This work indicates that Ece1 is a virulence factor of *C. albicans* and that it plays a critical role in mucosal *C. albicans* infections.

SU02/04

An efficient gene deletion strategy in *Mycosphaerella graminicola* using a non-homologous end-joining mutant strain

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Mycosphaerella graminicola (anamorph *Zymoseptoria tritici*) is an ascomycete fungal pathogen that causes Septoria tritici leaf blotch in wheat (*Triticum aestivum*). This host specific pathogen is the second most economically important disease of wheat with annual crop yield losses up to 50%.

As yet, the genetic basis of *M. graminicola* pathogenesis is not fully understood due to the lack of high throughput functional genomic tools. The low homologous recombination (HR) frequency in *M. graminicola* is a major factor limiting the elucidation of gene function through reverse genetics. Increased HR frequencies were achieved by the deletion of the *KU70* gene, which is involved in DNA break repair through the nonhomologous end-joining pathway. Growth and the virulence of the Δ*ku70* strain were comparable to that of the wild type strain IPO323. The effect of *KU70* gene deletion on HR frequency was investigated by deleting the *PyrG* gene, encoding for orotidine 5'-phophate decarboxylase (involved in uracil biosynthesis pathway). Results showed that the gene targeting efficiency increased to 70% in the Δ*ku70* strain, as compared to <5% in the wild type strain IPO323. This data confirms that the Δ*ku70* strain can be used as recipient for large-scale gene function analysis using reverse genetics.

SU02/05

***Dictyostelium discoideum*: a model for testing novel inhibitors of urokinase-type plasminogen activator**

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The social amoeba *Dictyostelium discoideum* is a useful non-animal eukaryote for testing novel compounds and dissecting cell regulatory molecular networks. We have used this social amoeba to test the effect of a series of arylboronic acids and pinacol esters on development, chemotaxis and viability of *Dictyostelium*. The compounds were investigated previously by collaborators for serine proteases and urokinase-type plasminogen activator (uPA) inhibition, both *in vitro* and *in vivo*. In those biochemical assays, three compounds, BC11, SR3 and

BC57, displayed micromolar (50 μM) inhibition of uPA with an excellent selectivity profile over related proteases (Smith *et al*., 2012). Notably, the same compounds disturbed cell adhesion and migration in *Dictyostelium*, without any effect on viability. Compound BC11 was chemotoxic rather than just chemostatic, proving to be the most potent and selective inhibitor of uPA in independent *Dictyostelium* and biochemical models.

Smith E., Spencer J., Ali M., Abdinejad M., Kanlanala J., Fishwick C. & Philippouu H. (2012) Elucidating novel urokinase-type plasminogen activator inhibitors. In *Journal of Thrombosis and Haemostasis*, pp. e10-e24.

SU02/06

Morphogenesis in *C. albicans*: shaping the cytokine signature

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Candida albicans is a human opportunist pathogen and can grow as yeast, pseudohyphae or true hyphae depending on environmental conditions. This morphological flexibility is considered as an important virulence factor facilitating *C. albicans* in its adhesion to host cells, invasion of tissue and escape from immune cells. Previous studies focused on the role of yeast and hyphal formation in *C. albicans* pathogenesis however the role of pseudohyphae in virulence and immune recognition has not been carefully assessed. This is a significant gap in our knowledge since most *Candida* species form pseudohyphae rather than true hyphae. Here we show that morphogenesis affects *C. albicans* cell wall composition and consequently cytokine induction. *C. albicans* hyphae induced the production of significantly less cytokines than yeast cells and hyphal cells were able to suppress the cytokines responses associated with the normal recognition of yeast cells. Pseudohyphae had intermediate physiological and immunological properties between those of yeast cells and hyphae in term of cell wall composition and cytokine stimulation. Mutants that were unable to form filaments stimulated an increased cytokine response when grown in pseudohyphal or hypha inducing conditions. In addition, *C. albicans* pseudohyphae were are able to suppress cytokine response induced by yeast cells.

SU02/07

Communication between *Candida albicans* and *Pseudomonas aeruginosa*

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Despite the extensive utilisation of drugs and vaccines, infections remain a leading cause of human mortality and morbidity worldwide. There is now evidence that some infections, for example of the cystic fibrosis (CF) lung, burn wounds or medical devices, are polymicrobial in nature. Our research concentrates on signal-mediated interaction between the fungus *Candida albicans* and the bacterium *Pseudomonas aeruginosa*. These microbes display a remarkable ability to adhere to abiotic surfaces and form biofilms on implantable medical devices. Interestingly, recent studies have demonstrated that these microbes can interact directly and indirectly with important consequences for expression of virulence traits. We showed that bacterial supernatants can inhibit fungal biofilm formation and are now investigating fungal pathways involved in the response to the bacterial signal(s). Libraries of protein kinase and transcription factor mutants are being screened via biofilm assays to identify the key signaling pathways governing fungal response. A better understanding of the inter-species cross-talk will shed light on the relevance and importance of these interactions during infection. Also, the identification of the fungal pathways that are affected by bacterial secreted molecules may lead to new opportunities for antifungal treatments.

SU02/08

Evolutionary rewiring of ubiquitination targets in *Candida albicans* promotes metabolic flexibility in host niches

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Candida albicans is a major human fungal pathogen that encounters nutritionally diverse host niches during infection. While *C. albicans* is considered a Crabtree-negative yeast (respiring in the presence of glucose), exposure to glucose down-regulates key metabolic transcripts involved in the utilisation of alternative carbon sources similar to the Crabtree-positive yeast, *Saccharomyces cerevisiae*. However, *C. albicans* has undergone significant rewiring in glucose-mediated posttranslational regulation as these encoded metabolic enzymes are retained. This allows simultaneous assimilation of alternative carbon sources. In contrast, in *S. cerevisiae*, simultaneous carbon assimilation is prevented by catabolite inactivation. We have shown that certain enzymes, such as isocitrate lyase (Calcl1), lack critical ubiquitination sites compared to *S. cerevisiae* Icl1 (Sclcl1) that mediate this catabolite inactivation. Interestingly, the components of the Glucose-Induced Degradation (GID) complex, which is responsible for targeting these enyzmes for degradation in *S. cerevisiae*, are conserved in *C. albicans* and appear to be functional, as evidenced by the rapid glucose-signalled degradation of Sclcl1 when expressed in *C. albicans*. Thus, while aspects of carbon assimilation are conserved in *C. albicans* compared to *S. cerevisiae*, there has been significant rewiring of ubiquitination targets. This resulting metabolic flexibility could play an important role in virulence *in vivo*.

SU03
Impact of bacteriophage in the environment

SU03/01

Phage resistance is costly for *Pseudomonas syringae* infections in the plant environment

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The evolution of bacterial resistance to phage infection and lysis is often metabolically costly in the absence of phages. Such costs have been quantified in nutrient-rich growth conditions but have yet to be measured in the plant environment.

We compare densities of *Pseudomonas syringae* pv. tomato DC3000 in its tomato plant host with multiple mutant strains that have acquired phage resistance mechanisms. Costs and benefits of phage resistance are assessed in the absence and presence of infective phages to which resistance evolved.

The ancestral bacterial genotype was exposed to 6 different phage strains and 1 spontaneously resistant colony was selected per treatment. Plants were then inoculated with phage resistant strains and co-inoculated with the relevant phages. Growth was measured by serial dilution colony counts of homogenised leaf tissue.

We found significantly reduced growth of mutant strains relative to the sensitive ancestral genotype. Interestingly, this growth was

not affected by the presence of phages inoculated concurrently. This suggests that phage resistance mechanisms are generally costly and that this could regulate diversity in the phyllosphere, reducing the ubiquity of phage resistance despite high abundance of phages. Moreover, phages may regulate bacterial population dynamics but have little effect on overall densities.

SU03/02

Analysis of typing phages from the *Escherichia coli* O157 typing scheme using whole-genome sequencing to classify and understand resistance and susceptibility

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Phage-typing is a widely used method for population analysis of *E. coli* O157 outbreaks but little is known about the mechanisms of interaction between the bacteria and the typing phages or the reasons why certain strains of *E. coli* O157 are resistant to particular bacteriophages. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) in bacteria can provide resistance to bacteriophages. Whole genome sequencing of 21 strains of *E. coli* O157including 8 phage types, and 12 typing phages (1 T7 and 11 T4 phages) used in the phage typing scheme of *Escherichia coli* O157 were analysed, using bioinformatics tools, to look for the presence of protospacers in typing phages which may correspond with CRISPR regions in resistant phage types. Comparison of the typing phage sequences revealed 3 different groups of largely homologous T4 phages. Homogenous and heterogeneous regions within the groups were analysed using bioinformatics tools and visualised using circos to reveal areas of genetic variation that could inform susceptibility patterns to phages of *E. coli* O157. Typing phage DNA was observed in certain strains of E. coli O157 but did not correlate with any particular PT. Other mechanisms of phage resistance are being explored.

SU03/03

Evolution of RDX degrading capabilities amongst aerobic bacteria

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Hexahydro-1,3,5-trinitro-1,3,5-triazine (Royal Demolition Explosive, RDX) is a toxic xenobiotic compound which has polluted soils for around seventy years and is a particular problem on military training ranges. Structurally, RDX has three nitramine groups on a six-membered saturated ring, an arrangement not yet been found in nature. A number of Actinomycetales (*Rhodococcus*, *Microbacterium* and *Williamsia* sp.) with RDX-degrading ability have been isolated from different countries including the United Kingdom, Israel, Ukraine and the United States. In all cases so far, the enzyme responsible was found to be XplA; an unusual cytochrome P450 with a fused flavodoxin domain. We have now shown that xplA is a part of larger genomic island, approximately 14 kb in size, which is highly conserved among all RDX-degrading species isolated to date. This high level of conservation suggests that the RDX-degrading capacity has been recently acquired, and distributed by horizontal gene transfer. The evolutionary origin of xplA is not clear; the closest homologue of xplA in a non-RDX degrading bacterium, *Gordonia terrae* shares only 46% sequence identity with XplA. *G. terrae* does not have any activity towards RDX, or the structurally similar compound HMX, and studies to understand and evolutionary origin of RDX-degradation are ongoing.

SU03/04

Comparison of temperate bacteriophages isolated from *Pseudomonas aeruginosa* isolated from non-cystic fibrosis bronchiectasis patients

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Non-CF bronchiectasis (nCFBR) and other chronic obstructive lung diseases are caused by localised inflammation and constriction of the bronchi of the lung. Inflammation and the production of thick mucus, similar to CF, is difficult to clear and creates an ideal environmental niche for opportunistic bacteria to colonise. The presence of *Pseudomonas aeruginosa* (PA) in chronically infected lungs correlates with loss of lung function. Temperate bacteriophages are viruses that infect and subvert their bacterial host through integration into the bacterial chromosome (prophage) and can express moron genes that offer a selective advantage to the infected host (lysogen) within these microenvironments. Prophage can have significant impact on the host by increasing: antimicrobial resistance, bacterial fitness; and may modulate immune responses to the bacterium. This study compares the host range of these temperate phages in nCFBR, and their ability to infect both a CF and nCFBR PA background. This study shows there may be evolutionary distance between the phages from CF PA and BR PA as there is a significant difference in infection profiles between nCFBR PA phages compared to those chemically induced from CF PA isolates. In addition, current vasodilatory therapies influence the induction or suppression of these prophages.

SU03/05

Survival strategies of *Pseudomonas aeruginosa* is dependent on its phage-like R-pyocins in the face of true competition

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Pseudomonas aeruginosa is a ubiquitous organism found in the environment. It has however gained prominence as a nosocomial pathogen especially in chronic lung infections of cystic fibrosis patients. It thrives in biofilms and produces pyocins which are bacteriocins that kill closely related strains. Five isolates identified in this study produced both S- and R-pyocins and produced different pyocin resistance and sensitivity profiles against one another. One Isolate, A026 produced pyocins that killed all four other strains using biological activity assay on agar plates but was also sensitive to the pyocin of each of the other strains. This reciprocated activity was due to the presence of protease resistant non-soluble phage-like pyocins. This was confirmed as R-type pyocins by the generation of deletion mutations in the R-pyocin gene locus of each of the strains. The mutants showed loss of the reciprocated biological activities. Confocal microscopy and biofilm studies of mixed cultures revealed that A026 was the dominating strain in both liquid culture and biofilm populations containing one of each of the other strains. Studying the dynamics of pyocin production and activity showed that these properties commenced at early exponential phase and peaked at late stationary phase.

SU03/06

Spontaneous temperate phage induction as evolutionary selection in host *Pseudomonas aeruginosa* isolated from cystic fibrosis and non-cystic fibrosis bronchiectasis patients

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Temperate phages play key roles in polymicrobial infections through the co-evolution and adaptation alongside the bacterial host and dissemination through horizontal gene transfer. Co-evolution is a continuing arms race between bacterium and phage. Cystic Fibrosis (CF) and bronchiectasis (BR) are chronic obstructive lung diseases, characterised by inflammation of the bronchi and the production of thick mucus which provides an ideal niche for colonisation by bacterial pathogens e.g. Pseudomonas aeruginosa (PA). Temperate phages also encode a myriad of genes with unknown functions. The aim of this study was to characterise spontaneous induction rates of temperate phages from PA during the growth cycle. 94 well-characterised PA isolates from patients with CF or BR were collected at the Freeman Hospital, Newcastle upon Tyne. Samples were equilibrated for cell density and plated on soft agar as a plaque assay and also evaluated in broth culture. Over a third of isolates showed spontaneous induction of temperate phages at early-growth phase and no induction was found in the stationary growth phase This research has identified a phenotypic trait genetically encoded by the phage to evade stationary phase growth and biofilm formation which may limit horizontal gene transfer.

SU03/07

Mass spectrometric analysis of marine bacteriophage ϕTW-1 infecting *Pseudoalteromonas phenolica*

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Genomic sequence and electron microscopic characterisation of Pseudoalteromonas phenolica phage TW-1 has been reported (Shin, H., Lee, J.-H., Ahn, C.S., Ryu, S, Cho, B., Archives of Virology, in press). It was a 39,940 bp siphovirus with a 73 nm capsid and large globular lateral projections at the tail tip. Here we report an analysis of the TW1 virion proteins by gel-HPLC-ESI-tandem mass spectroscopy on a Thermo Fisher LTQ Orbitrap Velos mass spectrometer. Fifteen virion proteins were identified. Unexpectedly, eight of these were found to wholly or mostly migrate in SDS-PAGE as a high molecular weight complex. Putative functions within the complex included the central tail fiber, tail end proteins, an extracellular polymer binding protein, and the major tail tube protein. Putative functions predicted in protein migrating as dissociated monomers included the tape measure, tail lysozyme, portal and major head protein. The reason for some of the tail proteins to fail to disaggregate in SDS is unknown. The second most abundant protein is found in the genomic position normally occupied by scaffold protein. An abundant scaffold protein remaining in the mature virion may explain the relatively large virion for a genome of this size.

SU03/08

Complete genome sequence of marine bacterium *Pseudoalteromonas phenolica* bacteriophage TW1

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Pseudoalteromonas phenolica phage TW-1 was isolated from a salt pond, South Korea. Electron microscopic characterisation indicated that it had a siphoviral morphology with 103 nm non-contractile tail and 73 nm capsid. The tail tip was decorated with an unusual globular structure. The phage TW1 genome was circularly permuted, and consists of 39,940-bp length double-stranded DNA with GC content of 40.19%. It was predicted to have 62 open reading frames and they were classified into functional groups such as phage structure, packaging, DNA metabolism, regulation, and additional function. No other phages were similar at the nucleotide sequence level. The most closely related phages at the protein sequence level were Salmonella phage E1, Vibrio phage pYD21, and related phages and prophages mostly found in Enterobacteriaceae. The phage life style prediction using PHACTS showed that it may be a temperate phage. However, genes related to lysogeny and host lysis were not detected in phage TW1 genome. This is the first report of P. phenolica -infecting phage and this phage genome study would provide useful information for further molecular research of P. phenolica host and its phage as well as their interactions.

SU03/09

The genotypic and phenotypic impacts of Shiga toxin-encoding bacteriophage interactions with their host cells

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Enterohaemorrhagic *Escherichia coli* (EHEC) are food borne pathogens that have become a worldwide public health concern during the last two and a half decades. Infection can result in haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and/or thrombotic thrombocytopenic purpura (TTP). The severity and life-threatening nature of EHEC diseases set them apart from other agents of bacterial gastroenteritis and underpin our need to develop better understanding of their emergence and control. The major virulence determinant of EHEC is Shiga toxin production (Stx), which is encoded on temperate lambdoid bacteriophages (Stx-phages) whose genomic organisation and regulatory functions are lambda-like. However, our model Stx phage, $\Phi 24_B$, differs from λ in several aspects, especially its ability to superinfect a single host cell. Furthermore, Stx-phage genomes can be as much as 50% larger than λ, and most phage genes encode proteins of unknown function. Through transcriptomic analysis, we aim to characterise the Stx phage-borne genes expressed in lysogen cells and assess the impact that Stx phage carriage has upon the bacterial host. The transcriptomes of *E.coli* MC1061 and its isogenic lysogen MC1061/$\Phi 24_B$ have been sequenced before and after phage induction and the analysis is revealing the presence of significant and informative differences in gene expression.

SU03/10

Understanding *Clostridium difficile* and its bacteriophages from the environment

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Clostridium difficile is a highly virulent gut pathogen causing nosocomial diarrhoea with over 13,352 reported cases in UK trusts during 2012. *C. difficile* has been isolated from a number of environmental sources such as soil, river, sediments and seawater, beside the isolation from hospital environment.However, it is not clear if these strains are actively replicating in sediment, or they exist as spores derived from sewage contamination. This project aims to understand the biology of *C. difficile* outside the human host, as this issue has not been addressed before. *C. difficile* isolates from hospital and other environments were grown under typical estuarine. Since the environment is considered as an ideal source of this pathogen and its phages; a collection of environmental strains and lytic phages were isolated from soil and sediment samples from the Middle East as the occurrence of *C. difficile* and phages in the general environment are lacking in that area. Phages will be morphologically and genetically characterised, with emphasis to investigate potential activity that may be able to be used as therapeutic agents in attempt to treat *C. difficile* infections. Identification and characterisation of the isolates is underway explain possible factors responsible for the geographical differences.

SU03/11

Isolation and characterisation of three novel bacteriophages of *Proteus mirabilis*

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Infection with Proteus mirabilis complicates the care of patients undergoing long-term bladder catheterisation. It results in the formation of crystalline biofilms within the catheter leading to blockage. With no strategy effective in preventing this, a significant level of patient morbidity is associated with catheterisation. The utilisation of bacteriophages to control bacterial infections has been postulated since their discovery almost a century ago, but few studies have explored their potential for control and prevention of urinary catheter-associated infections.

Here we describe the isolation and initial characterisation of three P. mirabilis phages. A library of 48 P. mirabilis clinical isolates was used as potential hosts with which to isolate phages from sewage. The host library was analysed using PFGE restriction endonuclease banding patterns to determine their relatedness to one another. Phages were isolated using standard enrichment techniques. The three resultant isolated phages' genomes were analysed by PFGE to confirm they were distinct phages. Transmission electron microscopy was used to determine phage size and morphology and SDS-PAGE was used to compare phage structural proteins.

Future work will apply the phages to in vitro catheterised bladder models to determine whether they can impact P. mirabilis colonisation and encrustation processes.

SU03/12

Comparision of viruses in two Scottish soils and relationships to soil physiochemical characteristics

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Viruses were purified from two contrasting Scottish soil samples: Machair (a coastal soil type unique to the west of Scotland) and Brown Earth. The relative abundance and diversity of the viruses present in the soil samples was characterised by electron microscopy and genomic sequencing. A wide variety of virus morphotypes were identified and these were found to be much more abundant in the Machair soil than in the Brown Earth. The abundance of different virus types was related to various physiochemical characteristics of the soil types. Nucleotide sequencing of genomic DNA from the virus samples has confirmed the data on identification of virus types in the soil samples obtained by electron microscopy and has indicated further differences in the abundances of virus types present in the different soils.

SU03/13

Combating an infectious misconception: phage, viruses, scale, size and the public

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The 'public' is often presented with the word 'virus' by the media, often erroneously. The term is typically associated with disease and infection. Images presented may be attractive pictures of bacteriophage, or fairly uninformative false-coloured blobs. Additionally, although people are aware of the small size of viruses, it is likely they do not understand the true scale and size of a virus particle.

'The very small world of viruses' was a public engagement event (delivered on World AIDS Day 2012) which attempted to demonstrate the relative size of viruses, their importance and uniqueness, and key messages regarding vaccination and treatment. The event was hands-on and included making a (plasticine) virus to infect a large (3x3m) cell, swabbing and examining cheek cells (to demonstrate relative size) and making a Christmas tree virus decoration (spiked polystyrene ball) to take home. The event attracted over 200 participants in four hours. Participation was monitored numerically (models made, pictures drawn), and ongoing discussions and verbal feedback were very positive, indicating that key messages were being understood.

Future developments will include improved management of visitor movement through the activities, focused conversation, additional representations of scale and enhanced capture of feedback for evaluation purposes.

SU03/14

Characterisation of Stx-phage and BamA interaction

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Shiga toxigenic *E. coli* (STEC) cause several outbreaks per year resulting in potentially life-threatening cases of HUS and TPP. Shiga toxin (Stx) genes are horizontally transferred to susceptible hosts by bacteriophages (phages), which increase the host's pathogenic profile. Phages can also infect gut commensal flora during STEC infections, which is thought to increase the severity of the resulting disease. An unexpectedly common infection strategy used by 70% of Stx-phages involves adsorbing to the host cell via BamA, an essential outer-membrane protein. BamA is highly conserved across *Enterobacteriaceae*, and this infection strategy has likely used BamA as the fundamental constant that has driven the dissemination of *Stx* and the emergence of high-profile STEC, *e.g.* O104 and O157. This essentiality makes BamA tricky to modify, but by fusing the membrane associated domain from the BamA orthologue of *Pectobacterium*

carotavorum, a chimera was generated that maintained BamA function whilst eliminating susceptibility to Stx-phage infection. The BamA epitopes supporting Stx phage adsorption were identified using a series of natural and synthetic BamA mutants. It's hoped this information will help us understand how Stx-phages recognise potential hosts. This knowledge might also improve our ability to limit Stx-phage infections for improved clinical outcomes.

SU03/15

Does phage subversion by temperate bacteriophages alter host bacterial physiology?

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Chronic lung infections such as Cystic fibrosis (CF) and non-Cystic fibrosis bronchiectasis (nCFBR) drive bronchial inflammation and the production of mucus that is difficult to clear by the normal mucocilliary action of the lung. Opportunistic bacteria including *Pseudomonas aeruginosa* (PA) can chronically infect the lung; deviation from a normal vegetative state to a state promoting biofilm formation within the mucus layer, creates a microbial infection that is difficult to treat clinically. Multiple 'deep sequencing' and 'community analysis' studies of these polymicrobial infections, associated with CF and nCFBR, have identified a core microbial community that is fairly stable and thus, does not truly explain the exacerbation in symptoms that can be potentially life threatening. PA is almost always found within biofilms in respiratory diseases and correlates with reduced lung function in chronically infected patients. Temperate bacteriophages subvert their bacterial hosts and encode moron genes that can alter cellular physiological function when expressed by the host. This study compares the metabolic changes in PA, when bacteriophage lysates isolated from patients with CF/nCFBR are used to infect PA isolates.

SU05
Pathogen genomics – current clinical applications

SU05/01

Whole-genome sequencing for *Stenotrophomonas maltophilia* outbreak investigation

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Stenotrophomonas maltophilia, which is intrinsically resistant to multiple antibiotics, is increasingly isolated from vulnerable patients. We investigated a possible outbreak in the Queen Elizabeth Hospital Birmingham by whole-genome sequencing nine *S. maltophilia* isolates from five patients, using Illumina MiSeq. Core proteome was identified from the sequenced isolates and seven web-accessible whole *S. maltophilia* genomes. Comparison of core proteomes, each consisting of 2058 coding sequences, revealed no cross-infection events or recent common source. Previously unidentified mixed infection was observed in one of the patients. In addition, whole-genome sequencing revealed the presence of a number of putative pathogenicity factors, including a prophage-encoded virulence factor present only in one isolate. The information obtained in this study was used to obviate the need for infection control investigation. The study supports the growing belief that whole-genome sequencing is a useful tool for detecting or disproving outbreaks and could be eventually used routinely in the hospital setting.

SU05/02

Outbreak or not: genomic investigation of an abrupt increase in ESBL *E. coli* bacteraemia cases in a large tertiary health care facility

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The last 12 months have seen a rise in the number of bacteraemia cases caused by ESBL positive *E. coli* in the NUH NHS trust. To investigate this we characterised 140 *E. coli* bacteraemia isolates over a five month period. To contextualise our findings we compared them to 125 *E. coli* from urine samples over the concomitant time frame. ESBL carriage in the bacteraemia population was significantly higher. MLST of the bacteraemia population showed two main STs with ST131 being the most dominant and significantly higher in prevalence than the urine population, followed by ST73.

Genome sequencing was performed to investigate if there was an "outbreak" of ST131 and ST73 ESBL *E. coli* causing bacteraemia in the hospital. The ST131 strains show very little variation as previously reported, but SNP variation suggests spread of an outbreak strain is unlikely. ST73 strains show enormous diversity, also ruling an outbreak as extremely unlikely. Together this data set suggests an increase in bacteraemia due to selected ESBL *E. coli* sequence type strains in NUH NHS is not due to an outbreak as implied from antibiogram and MLST data, but rather an increase in the prevalence of such strains in the community.

SU05/03

Cholera microbiology in the context of vaccination, safe water, sanitation, and clinical care

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Oral cholera vaccine (OCV) has recently been embraced by the World Health Organisation, and relief agencies aim to integrate vaccination into their emergency responses. Modeling human populations theorises that OCV will suppress disease transmission and avert deaths, however estimates of the basic reproductive number of *V. cholerae* in Haiti ranged from *R0* = 1.06 (Nippes) to 2.63 (Artibonite); and in Zimbabwe from *R0* = 1.11 (Mashonaland East) to 2.72 (Matabeleland South); specifying vaccination coverages ranging from 6.9%–81.0%. Uncertainty in modeling in Haiti is attributed to poor knowledge reflected in values spanning 5 orders of magnitude accorded to key microbiological parameters including microbial dynamics in shedding and the aquatic reservoir, infectious dose, concentration, and the hyper-infectiousness of freshly-shed stool. Little is known about the environmental cycle, the dynamics of lytic phage, and drivers of microbial evolution and proliferation in the contrasting settings in which cholera occurs. Additional uncertainty pervades the quality of case reporting, the dynamics of individual and population immunity, indirect effects, the vagaries of the weather and the long-term impacts of all interventions on microbial selection. Cholera vaccination is a significant advance, but our ignorance in specific areas of V. cholerae genomics and physiology makes the adversary formidable.

SU05/04

Investigation on the effect of quorum sensing on swarming of *Aeromonas caviae*

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Aeromonas are ubiquitous water-borne bacteria. In humans gastroenteritis and wound infections are associated with *Aeromonas*. Expression of lateral flagella is likely to be a significant virulence determinant for the *Aeromonas* strains. Previous studies have suggested that quorum sensing (QS), is contributed to swarming of some other bacteria. There is no available study on the effect of QS on swarming of motile *Aeromonas*. We aimed to investigate this effect by knocking out the cognate genes and observation of any probable swarming changes. A Δ*ahyIR* mutant strain was constructed by insertion of kanamycin (Km) cassette at the middle of the QS cognate genes producing a double mutation in *ahyI* and *ahyR* in a time. The mutant gene then cloned into a suicide plasmid and was introduced to *A. caviae Sch3N* as a swarming positive *Aeromonas* strain. Double homologous recombinant mutant strain was employed for investigation of the swarming on the swarming plats and phenotype changes was compared with the wild type. The results showed that the *ahyIR* defective strain had swarmed on a solid surface in a similar way of the wild type. It is concluded that AhyI-AhyR QS signaling doesn't plays a major role in control of the *A. caviae* swarming.

SU05/05

Tool for Rapid Annotation of Microbial SNPs (TRAMS): a simple program for rapid annotation of genomic variation in prokaryotes

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Next-generation sequencing (NGS) has been widely applied to a variety of microorganisms for evolutionary and epidemiological investigations. Genome wide variation (single nucleotide polymorphism, SNP; multiple nucleotide polymorphism, MNP) from NGS data analyses need functional annotation for their impact on coding sequences. We have developed a program, TRAMS, to annotate large numbers of SNPs between several strains. TRAMS needs a tab delimited text file containing SNP locations, reference nucleotide and SNP alleles along with a reference genome in GenBank/EMBL format. SNPs are annotated as synonymous, nonsynonymous or nonsense. Nonsynonymous SNPs in start and stop codons are separated as non-start and non-stop SNPs, respectively. SNPs in multiple overlapping features are annotated separately for each feature and MNPs within a codon are annotated together. TRAMS is especially designed for growing number of NGS users with limited computational experience who can use an executable file on WINDOWS computers (http://sourceforge.net/projects/strathtrams/files/Latest/) without needing any installation or command lines to run. TRAMS is available as a Python script for MacOS and Linux users and also works within Galaxy, a highly used tool for analysing NGS data. TRAMS accurately annotated >70,000 SNPs between four Corynebacterium diphtheriae strains and will be very useful in analysing microbial genomic diversity.

SU05/06

Replacement of *Candida glabrata* IME1 and IME2 with their *Saccharomyces cerevisiae* ortholgues results in a functional sexual cycle

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Many fungi are thought to reproduce exclusively through asexual mitotic division. This has come into question as population structures suggestive of genetic recombination; genes encoding proteins with no known function outside of mating or meiosis and cryptic sexual cycles have been revealed. *Candida glabrata*, a human fungal pathogen, has a mating type locus and contains orthologues of *S. cerevisiae* involved in mating and meiosis, yet no evidence of sexual reproduction exists. In this study, we show that *C. glabrata* can mate and produce progeny that undergo genetic recombination. *C. glabrata IME1* and *IME2* encode proteins that do not complement mating and sporulation defects of *S. cerevisiae* Δ*ime1* and Δ*ime2* mutants. Replacement of these genes in *C. glabrata* with their *S. cerevisiae* orthologues results in mating competency. Mating gave stable *C. glabrata* diploids that sporulated, producing four celled tetrad-like structures, which yielded haploid cells and a 2:2 segregation of markers. *De novo* genome sequencing of both parental strains and the four progeny identified regions of recombination in the progeny. Our results are indicative of that in addition to the loss of orthologous mating/meiosis proteins, those retained may have had their function reassigned, resulting in a reliance on asexual reproduction

SU05/07

Comparison of the networks of orthologues between two closely related species, *S. cerevisiae* and *C. glabrata*

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Candida glabrata is an opportunistic pathogen however little is known about its mechanism of virulence. Phylogentically *C. glabrata* is most closely related to the non-pathogen *Saccharomyces cerevisiae*. Thus, a common assumption made between the two species is that orthologues share a similar function, protein-protein interaction (PPI) profile, and genetic interactions. Here we show that this is not true even between two very closely related species. Firstly, we examined the simple assumption that the higher the conservation of Protein A and Protein B pairs between *C. glabrata* and *S. cerevisiae*, the greater the conservation between the two networks. Initial interactions for Yeast-2-Hybrid (Y2H) screening of *C. glabrata* proteins were selected based on orthologue similarity, how well PPI covariates are associated to reported *S. cerevisiae* interactions, and whether they form part of the same system. We show that transferability from protein affinity networks to Y2H is poor but that transferability between Y2H networks in yeast is relatively high. Next, we examined the highly conserved Glycerol-3-phosphate dehydrogenase (Gpd1/2) enzymes in *S. cerevisiae* and *C. glabrata* and show not only that the PPI and genetic interaction profiles of the two species differ, but also that there are functional differences in response to osmotic stress.

SU05/08

Molecular diagnostics to identify pathogens in culture negative neonatal sepsis

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It is estimated that in up to 80% of neonates treated for sepsis, blood cultures fail to grow an organism. This study investigates

organisms present in culture negative neonatal early (EOS) and late onset sepsis (LOS) by molecular methods. A series of tests, including a panel of bacterial specific duplex real time qPCRs, 16S rDNA broad range qPCR, Candida spp multiplex qPCR, viral microarray and metagenomic sequencing are used to process culture negative blood samples of 550 infants with suspected LOS and 250 with suspected EOS from two separately a conducted studies.

Here we present preliminary results from the organism specific and broad range real time quantitative PCRs on samples of infants with EOS. A large proportion of samples were positive for ubiquitous bacteria such as coagulase negative Staphylocoocus (70%), *Enterobacteriaceae* (45%) and *Staphylococcus aureus* (20%), often these were present together. S. pneumoniae was detected in 8%, Enterococcus in 5%, Group B Streptococcus in 4%, Ureaplasma in 4% and Group A Streptococcus in 1%. PCRs include relative quantitations for estimation of the bacterial load. These will be compared to clinical data in an attempt to establish threshold values to distinguish possible sample contamination at source from true pathogenesis.

SU05/09

Investigating pathogenesis and virulence of the human pathogen, *Vibrio vulnificus*

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V. vulnificus is a Gram negative, lactose fermenting, halophilic rod shaped pathogen that is found predominantly in estuarine environments. The bacterium is an emerging pathogen, first reported by the US Centre for Disease Control in 1979. V. vulnificus is divided into 3 main biotypes; biotype 1, associated with human infection; biotype 2, causing disease in eels and biotype 3, a hybrid of both biotypes 1 and 2. Biotype 1 strains are the main focal point of interest in this study and are highly variable in their pathogenicity potential. A number of attempts have been made to group isolates into clusters with the aim of correlating these clusters with virulence potential. However, published typing methods have not been able to distinguish isolates with 100% accuracy.

The aim of this project is to use both a bioinformatic and wet lab approach to identify novel virulence factors in the hyper virulent strains that are not present in the lesser virulent strains. Bioinformatic data will be used to generate an informed list of virulence genes for targeted disruption. Data on the characterisation of such mutants will then be presented.

SU05/10

Molecular characterisation of the 2011 Group A streptococcal scarlet fever outbreak in Hong Kong

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Objectives A scarlet fever outbreak occurred in Hong Kong in 2011. The majority of cases resulted in the isolation of *Streptococcus pyogenes emm*12 with multiple antibiotic resistances. The main objective of this study is to examine the microevolution of the *emm*12 lineage associated with the outbreak.

Methods We sequenced the genomes of 57 *emm*12 scarlet fever outbreak isolates and 50 temporally and geographically matched *emm*12 isolates not associated with scarlet fever. A further 34 *emm*12 strains isolated up to five years prior to the current outbreak and three historically unrelated *emm*12 strains were also included.

Results Phylogenetic analysis suggests the outbreak was multiclonal. Novel mobile genetic elements distributed across the major lineages, include a 64.9 kb integrative and conjugative element (ICE) encoding tetracycline and macrolide resistance, and a 46.4 kb prophage encoding the superantigens SSA and SpeC, and the DNase Spd1. Phenotypic comparison of two outbreak isolates with the globally disseminated *S. pyogenes* M1T1 suggests that the mechanism of infection is unrelated to *covRS* mutation.

Conclusion The multiclonal nature of the *emm*12 scarlet fever isolates suggests factors such as mobile genetic elements, environmental factors and host immune status may have contributed to the 2011 scarlet fever outbreak.

SU05/11

Horizontally acquired glycosyltransferase operons drive salmonellae lipopolysaccharide diversity

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In order to ensure long-term survival in a mammalian host, bacterial pathogens frequently evolve mechanisms to vary the composition of their surface structures. *Salmonella* sp., cause severe infections in a range of mammalian hosts and guard themselves with a protective coat, termed the O-antigen, an constituent of the bacterial lipopolysaccharide. Through genome sequence analyses we found that *Salmonella* have acquired an unprecedented repertoire of glycosyltransferase operons for modifying their O-antigen coat. There is strong evidence that these genetic factors exhibit a dynamic evolutionary history driven by recombination and gene shuffling events leading to new gene combinations and are spread through the bacterial population by bacteriophage. In addition to this genetic repertoire, we determined that *Salmonella* can and often do employ stochastic mechanisms for expression of these genetic factors. This means that O-antigen coat diversity can be generated within a *Salmonella* population that otherwise has a common genome. The role attributed to bacteriophage in generating this diversity highlights that *Salmonella* are acquiring an extensive repertoire of O-antigen modifying traits that may enhance the pathogen's ability to persist and cause disease in mammalian hosts. Such genetic traits may make useful markers for defining new epidemiological and diagnostic tools.

SU05/12

The molecular epidemiology of multi-drug resistance organisms in companion animal species

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Multi-drug resistance (MDR) is a serious clinical problem in both human and animal care. With rising levels of use, reduced discovery and development of novel antimicrobial compounds and the rapid global dissemination of horizontally-acquired resistance, many clinical treatment protocols are vulnerable. Within the veterinary setting, there are increasing reports of clinical disease associated with MDR organisms in companion animal species, although the epidemiology of these infections is not well understood. Between 2006 and 2012, 19 MDR *E. coli* isolates from canine urinary tract infections were identified at the

Hospital for Small Animals R(D)VS. Phenotypic characterisation revealed that many were AmpC positive, and this was then confirmed genotypically with PCR. The isolates have been further characterised using next generation sequencing and compared with other published *E. coli* isolates in terms of their phylogeny, plasmid-acquired resistance and possession of virulence genes. Virulence potential of the isolates was also investigated using a *Galleria mellonella* infection model.

SU05/13

Isolation and NGS characterisation of *Cryptosporidium* clinical isolates

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Cryptosporidium is a protozoan parasite that causes prolonged gastroenteritis (2-3 weeks) in humans, mostly commonly young children. Whilst self limiting in immuno-competent hosts, severely immuno-compromised patients suffer severe, sometimes life threatening disease. Most human disease in the UK is caused by C. parvum or C. hominis.

Routine diagnostics are based on microscopy of stained smears or detection of antigens in stools for presence/absence of the genus. Although species-level typing is undertaken on samples referred to the Cryptosporidium Reference Unit, there is no accepted subtyping scheme for C. parvum and C. hominis. Only three Cryptosporidium species have been genome sequenced to date, and very little is known about the sub-types and diversity of Cryptosporidium species. This is complicated by the need to passage clinical isolates through animals to collect sufficient oocysts for subsqeuent genomic DNA recovery and sequence analysis.

Here we report on the use of immunomagnetic seperation to isolate 10^4 oocysts from clinical samples prior to disruption of oocysts, extraction of sub-nanogram quantities of genomic DNA, generation of Nextera XT sequencing libraries and NGS characterisation of the genomes of C.hominis and C.parvum. We also present the genome sequence of C. viatorum, a recently identified human pathogen.

SU05/14

Metagenomic technology as a tool for the discovery of novel antimicrobials

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The increasing emergence of antimicrobial resistance in bacterial pathogens has led to a demand for novel antimicrobial compounds. Metagenomic techniques allow the study of the whole microbiome (culturable and as yet unculturable bacteria), and as such play a major role in novel compound discovery. Indeed, the discovery of turbomycin from soil metagenome suggests that novel antimicrobial compounds can be identified using metagenomics. We prospected a rumen fosmid based metagenome library for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Salmonella typhimurium*, and *Enterococcus faecalis*. Metagenomic clones (2µl) were gently pressed onto a lawn of 500µl of a pathogen (OD_{600}=1) on Luria-Bertani agar plates. Zones of clearing around the clones, indicative of antimicrobial gene inserts, were observed in 255 of 8448 clones after overnight incubation at appropriate temperatures. These putatively positive inserts are now in the process of being pyrosequenced. Rumen metagenomic and pure culture genomic datasets have also been mined, using BLAST and BioEdit, for homology to known antimicrobial gene sequences obtained from NCBI BLAST. Numerous hits were identified across many antimicrobial classes, especially aminoglycosides, penicillins and carbapenems. These antimicrobial genes will be expressed in a host and fully characterised in order to determine their novelty and potential applicability.

SU06
Regulatory phosphate-based molecules

SU06/01

Analysis of a phosphoregulatory module for promoter selectivity in *Chlamydia trachomatis*

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Upon experiencing certain types of stress, the obligate intracellular parasite *Chlamydia trachomatis* is able to enter an alternative growth mode, termed the persistence phenotype. In this state, chlamydiae remain viable, but non-cultivatable, yet are able to re-enter normal development upon removal of the stress condition. How *Chlamydiae* sense conditions that are not suitable for growth, or subsequently transduce this signal into a global transcriptional modulation, is not clear.

The *Chlamydiae* maintain three sigma factors, of which two (σ^{28}, σ^{54}) are considered to be alternative to the standard housekeeping sigma factor (σ^{66}). *Chlamydiae* also encode homologues of a phosphatase/antagonist/kinase partner switching regulatory module (RsbU/RsbV/RsbW, respectively), which functions in the regulation of σ^B-mediated stress response in *Bacillus subtilis*. Here we extend the knowledge of the chlamydial Rsb regulatory module system, and report that in addition to its kinase activity (Hua *et al*., 2006), $RsbW_{Ct}$ is also an anti-sigma factor, the first reported in the *Chlamydiae*. We propose that the phosphorylation state of two antagonists, $RsbV1_{Ct}$/$RsbV2_{Ct}$, may play a role in the regulation of chlamydial promoter selectivity, and thus allow a mechanism for the induction of the persistence phenotype in a mechanism similar to that of the σ^B-stress response in *B. subtilis.*

SU07
Microbial survival in the host

SU07/01

Phospho-regulation of the *Candida albicans* hyphal repressor Nrg1 by the cell wall biosynthesis kinase Cbk1

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In the human fungal pathogen C. albicans, negative regulation of hyphal development is mediated by a repression pathway that involves the recruitment of the co-repressor Tup1 to the DNA by the DNA-binding protein Nrg1, the negative regulator of filamentous growth. Cells that lack Nrg1 are characterised by a constitutive filamentous phenotype.

This study assessed the possible phospho-regulation of Nrg1 by the cell wall biosynthesis kinase Cbk1, which is absolutely required for polarised growth. Three Cbk1 consensus target sites in Nrg1 were identified. Two of these Cbk1

phosphorylation sites are located in the DNA-binding domain of Nrg1, specifically at the end of each zinc finger motif of this protein. Our hypothesis is that Cbk1 phosphorylates Nrg1 at these three sites in order to modulate its DNA-binding ability and release the repression to allow the yeast to hyphae morphological switch.

We targeted the three Cbk1 consensus sites in Nrg1, and non-phosphorylatable and phospho-mimetic mutants were generated. The results show that the non-phosphorylatable mutant behaves like the wild type but shows a minor hyphal growth defect, while the phospho-mimetic mutant releases Nrg1 repression. The mutations at these sites abolished the binding of Nrg1 to the promoter of hyphal-specific genes leading to their derepression.

SU07/02

Microbial ecology of the sheep mammary gland microbiome

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Intramammary infections (IMI) in sheep present as clinical mastitis or subclinical infection detectable by a raised somatic cell count (SCC). IMI have a major economic impact through death, premature culling and reduced milk production. Over 130 species of bacteria have been associated with IMI in cattle [1] and there is no reason to consider that this number of species cannot infect the sheep mammary gland (MG). Given the inevitability of IMI, we hypothesise that the sheep MG might be a site where bacteria live in a microbial community with certain members affecting SCC.

Molecular and epidemiological techniques are being used to investigate the sheep MG microbiome. A longitudinal study of 380 samples collected over 8 weeks from thirty ewes is being conducted. Comparison of molecular fingerprints (DGGE) of milk samples in a mixed effects regression model indicates that 9 bands from PCR-DGGE analysis are significantly associated with SCC change, indicating that variation in SCC is associated with bacterial community composition. 454-pyrosequencing is being used to identify bacterial groups related to changes in SCC. Preliminary conclusions indicate a complex and dynamic bacterial community in the MG of sheep.

1. Watts, J.L. (1988). Etiological agents of bovine mastitis. *Veterinary Microbiology* **16**(1), pp.41-66.

SU07/03

Quorum sensing, protein synthesis and morphological transitions in *Candida albicans*

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Farnesol is a quorum-sensing acyclic sesquiterpene alcohol that inhibits the transition to hyphal/pseudohyphal growth in *Candida albicans*. Similar to cellular stress conditions, exposure to this alcohol causes a rapid inhibition of global protein synthesis leading to altered programmes of gene expression. In the related yeast *Saccharomyces cerevisiae,* fusel alcohols signal nitrogen scarcity to inhibit protein synthesis by targeting the translation initiation factor, eIF2B. A similar mechanism for the regulation of translation exists in *Candida albicans*, where fusel alcohols also induce pseudohyphal growth in a process that is associated with pathogenesis. A variety of cell biological and genetic assays suggest that in contrast to fusel alcohols, farnesol does not impact upon eIF2B activity. Rather, here we present experiments that suggest farnesol impacts upon the interaction of the mRNA with the small ribosomal subunit during translation initiation.

This work highlights that the inhibition of translation initiation in response to signalling molecules can lead to a diversity of phenotypic outputs. These may depend on both the mechanism of regulation and the assimilation of this mechanism with the other effects of the signalling molecule. Overall the integration of these responses may have implications in pathogenicity.

SU07/04

Understanding antibiotic susceptibility in urosepsis: transcriptomic response of *Escherichia coli* CFT 073 to ciprofloxacin challenge *in vitro* model and in a blood stream infection model

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Escherichia coli is the common cause of urosepsis. Understanding *E. coli* differential gene regulation to its varying environments might shed some light onto combating urosepsis. In an ongoing study, we compared *E. coli* CFT 073 response to ciprofloxacin in human whole blood, serum and Iso-sensitest (IST) broth. The antibiotic challenge was introduced at the mid logarithmic phase of growth of the organism to depict a better clinical scenario. Global gene expression profiling of these conditions was examined using strain specific microarray. Analysis showed genes are differentially regulated between IST, serum and whole blood. Ciprofloxacin challenge lead to further differences in gene regulations, notably in functions such as DNA replication, prophage assembly, and SOS response. The findings presented here provide insights into the metabolic and susceptibility characteristics of *E. coli* CFT 073 in bacteraemia. The project is the first transcriptomic analysis of this organism grown purely in human blood and serum. This knowledge will eventually reach a stage where biomarkers (genes that specifically change to urosepsis therapy) are identified and put to a clinical use.

SU07/05

Using a high-density transposon library of *Salmonella enterica* serovar Typhi and Vi bacteriophage infection to investigate the regulation of the Vi capsular antigen

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The regulatory mechanisms underlying the expression of the *Salmonella* Typhi cell surface-associated Vi polysaccharide capsule are complex and involve a significant degree of differential gene expression. Some of the two component systems implicated in this modulation have been identified (ie RcsB-RcsC, OmpR-EnvZ) as have many of the genes that are differentially expressed such as those of the Vi operon itself, the flagellin genes (ie. *fliF*) and a variety of SPI-1 type III secreted proteins (SipA, SipC).

We investigated the possibility that other regulatory networks would be involved in this differential expression using a combination of a high-density transposon (TraDIS) library of *Salmonella* Typhi strain BRD948 and a phage that targets the Vi

capsule, Vi phage type II-E1. Results identified not only a further regulatory component, BarA-SirA (involved in SPI-1 expression) but also a gene which is important in the cascade of genes responding to oxidative stress conditions, namely *oxyR*, and a gene that attenuates the activity of RcsB-RcsC, *yrfF*, also called *igaA* (Intracellular growth attenuator). A role for RcsA is also implicated in this intricate regulation of Vi capsule expression.

SU07/06

Manipulation of phagosome dynamics by the fungal pathogen *Cryptococcus neoformans*

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Cryptococcus neoformans is a lethal fungal pathogen of immunocompromised individuals, infecting approximately one million people worldwide per year, with an overall mortality rate of 60%. *C. neoformans* initially infects the human lung before disseminating to and infiltrating the host central nervous system, leading to cryptococcal meningoencephalitis. During cryptococcosis, macrophages are vital in the innate immune response. However, it is well known that cryptococci are able to replicate within the macrophage phagosome and escape the phagocyte in a non-lytic manner (vomocytosis). The molecular detail that regulates and allows this replication and escape is yet to be defined.

Furthermore, a detailed examination of the cryptococcal containing phagosome over the duration of macrophage infection has still not been completed. Previous observations of some late phagosome markers has led to the hypothesis that Cryptococci persist within a normal phagolysosome, rather than modifying the maturation processes. However, using a combination of immunofluorescence, live imaging dyes and fluorescently tagged proteins we now show that live, but not dead, Cryptococci subtly modulate phagosome behaviour to facilitate intracellular survival and proliferation.

SU07/07

Identification of a novel regulator required for stress resistance and virulence in *Candida albicans*

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Candida albicans is exposed to a range of stresses during phagocytosis by innate immune cells, including reactive oxygen species (ROS), cationic fluxes, and an acidic pH environment. Whilst certain regulatory proteins, including the Hog1 and Rad53 kinases and the Cap1 transcription factor, have been found to respond to ROS or cationic stress, there is still much to learn regarding how this major pathogen senses and responds to physiological relevant stresses. To address this we have performed quantitative fitness analysis to screen available *C. albicans* gene deletion collections for mutants which exhibit impaired growth in response to oxidative, cationic or pH stresses. This identified genes not previously implicated in stress responses, including the Pho4 transcription factor. Cells lacking *PHO4* were significantly more sensitive to oxidative, cationic and acidic pH stress compared to wild-type cells, and displayed attenuated virulence in a *Caenorhabditis elegans* model of infection. Current studies are directed at delineating the role of Pho4 in mediating *C. albicans* stress resistance and virulence. Significantly, the Pho4 homologue in *Saccharomyces cerevisiae* is not required for oxidative or osmotic stress resistance, indicating that the function of Pho4 has been reassigned in *C. albicans* to respond to the stressful environments encountered within the host.

SU07/08

Phase variable Cj0031 RM system in *Campylobacter jejuni* plays important role in adaptation of bacteria within host

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The genome of Campylobacter jejuni, a major cause of food-borne diarrheal disease, contains many surface genes with mononucleotide repeat tracts (PolyG/PolyC) in the reading frame. These genes undergo phase variation (ON/OFF switching) with important implications for survival and adaptation of bacteria. PV rates were determined using reporter construct for cj0031. Mutation rates of a G10 tract were 1.5 fold higher than a G9 repeat tract in cj0031. Methyltransferase activity of Cj0031 was analysed by Southern blotting leading to identification of a putative recognition site.

An investigation of functional abilities showed that mutation of cj0031 enhanced adhesion, invasion and biofilm formation without having any affect on the motility of C. jejuni. A marginal 5-fold phage restriction activity of Cj0031 was also detected. It is postulated that Cj0031 mainly controls the phasevarion of other genes in C. jejuni through methylation of target sequences located either in promoter/intergenic regions rather than having active involvement in protection of hosts from phages. The high PV rates of cj0031 might be compatible with its role as a phasevarion thereby facilitating the rapid adaptation of C. jejuni to the micro-environment of hosts.

SU07/09

Modeling of HBx and partner protein interactions

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Owing to its interaction with multiple host proteins, Hbx, a hepatitis B viral protein, plays a pivotal role in the modulation of host cell cycle, cellular proliferation and inhibiting apoptosis. In this study we have constructed *in silico* molecular models of HBx and its commonly known partner proteins such as protein phosphatase 2C and E4F1 transcription factor using multiple threading alignment and iterative fragment assembly simulation. The molecular models were assessed for their structural and thermodynamic stability. The selected model of HBx was used to construct the dimer conformation using Symm Dock. Finally molecular docking of HBx and its modelled (PP2C, E4F1) and structured (Damaged DNA Binding Protein) were undertaken to investigate the potential binding sites involved in the interactions. Structural conformation, interaction interfaces, free energy values and intermolecular hydrogen bond patterns were evaluated to explore the potential interacting regions. The results provide structural insights of HBx protein and its interacting partners which may not only facilitate in our understanding of HBx and Hepatitis B biology but also highlights the regions that could be exploited to develop any therapeutic interventions targeting HBx.

SU07/10

***Candida albicans* Ece1p is a putative pore-forming toxin that damages human epithelial cells**

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Candida albicans is a commonly encountered opportunistic fungal pathogen of humans. During hyphal growth, the ECE1 (**E**xtent of **C**ell **E**longation) gene is highly expressed. ECE1 encodes a 271 amino acid protein (Ece1p) which serves as a substrate for KEX2 protease-mediated cleavage in vitro. Cleavage of Ece1p yields eight separate peptide fragments, seven of which terminate in a lysine-arginine (KR) motif. In this study, we identify an internal 32 amino acid peptide sequence in Ece1p as the active region responsible for C. albicans damage in human epithelial cells in vitro. The ability to cause damage is dependent upon an intact C-terminal KR motif and the Ece1 peptide appears to form pore-like structures in host epithelium by scanning electron microscopy. We propose that Ece1p acts as a putative fungal pore-forming toxin, the first of its kind in a human pathogenic fungus.

SU07/11

The polarised growth switch in *Candida albicans* yeast buds is controlled by the action of Cdc28 on Lrg1

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The small GTPase Rho1 is the positive regulatory subunit of β-1,3 glucan synthase, responsible for the main structural component of the cell wall. In turn Rho1 is negatively regulated by Lrg1, its GTPase activating protein (GAP). A lrg1ΔΔ mutant constitutively forms highly elongated and invasive pseudohyphae. Using Exo84 as a marker, we show that the extended polarised growth is due to a failure to relocate the polarity machinery from the bud tip to the bud neck. CaLrg1 contains 5 complete motifs and an additional 15 minimal sites for phosphorylation by Cdc28/Cdk1. These sites are clustered in an N-terminal extension missing from the S. cerevisiae Lrg1 homologue. Consistent with this, GST-Lrg1 is phosphorylated in vitro by Cdk1. Substitution of the putative Cdk1 targets with non-phosphorylatable alanine has little phenotypic effect. However, phosphomimetic glutamate substitutions results in highly polarised growth similar to, but milder than the lrg1ΔΔ phenotype. Taken together these result suggest that the cessation of polarised growth of the yeast bud is mediated by Rho1 whose activity is controlled by the action of Cdc28 on Lrg1.

SU07/12

Identification of prophage-encoded sRNAs in Enterohaemorrhagic *Escherichia coli* O157:H7

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Enterohaemorrhagic Escherichia coli (EHEC) O157:H7 strains are associated with serious disease in humans resulting from the activity of bacteriophage-encoded Shiga toxins. Cattle are considered the primary reservoir of these EHEC strains and our work has shown that EHEC O157:H7 has a tropism for the terminal rectum of cattle. In collaboration, we have demonstrated that EHEC O157 strains containing Stx2-encoding bacteriophages are more likely to be associated with high excretion levels from cattle and also with serious human disease. Our research has focused on the how integration of Stx2-encoding bacteriophages can contribute to EHEC colonisation and impact on excretion levels. Our published work demonstrated that Stx2 prophages alter the expression of the bacterial type 3 secretion system that is important for EHEC O157:H7 colonisation of cattle. Here we have employed UV-crosslinking and high throughput sequencing of RNA complexes (CRAC) to identify the targets of Hfq-mediated RNA regulation in EHEC O157:H7 strain Sakai. The mapping identified sRNAs expressed from prophages, including a family of homologous sRNAs that inhibit housekeeping sRNAs; we have termed these 'anti-sRNAs'. One anti-sRNA expressed from adjacent to stxAB increased the translation of genes required for haem uptake and processing by restricting FnrS-mediated repression.

SU07/13

Thermotolerance in *C. glabrata* is regulated by a calcineurin-linked trehalose accumulation pathway

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C. glabrata is now reported to be the second prevalent yeast commensal pathogen after C. albicans. It is known for its high resistance to various stresses, for instance its high resistance to currently used antifungals. Calcineurin is a serine/threonine specific protein phosphatase, which regulates cell wall integrity, virulence and resistance to different stresses, including antifungal stresses. In S. cerevisiae, several of these phenotypes are mediated by the transcription factor Crz1p via STRE-containing genes. Recently, it has been found that C. glabrata calcineurin mutants have lower thermotolerance than the wild type strain, but the underlying mechanism of this phenotype is not clear (Ying-Lien Chan *et al.*,. 2012). In our study, decreased trehalose levels and increased neutral trehalase activities were found in the Cgcnb1 mutant compared to the wild type strain, after two hours heat challenge. Thermotolerance of the Cgcnb1 mutant is restored by knocking out the trehalase-coding gene NTH1. Our studies suggest a novel pathway of calcineurin-linked regulation on trehalase activity under high temperature stress.

SU07/14

Delineating the mechanism underlying the synergistic killing of *Candida albicans* by combinatorial cationic and oxidative stresses

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Following phagocytosis by innate immune cells, *Candida albicans* is subjected concurrently to reactive oxygen species and cationic fluxes. This is significant as such combinations of stresses are much more potent at killing this fungal pathogen then the corresponding single stresses. Strikingly, we find that the enhanced killing of *C. albicans* by simultaneous exposure to both H_2O_2 and cationic stress is due to stress-pathway interference. The normal transcriptional response to H_2O_2, mediated by the AP-1-like transcription factor Cap1, is abolished following combinatorial stress treatment. In response to H_2O_2, Cap1 is oxidised on conserved cysteine residues which masks a nuclear export sequence resulting in its nuclear accumulation and induction of Cap1-dependent genes. However, exposure of *C. albicans* to both H_2O_2 and cationic stresses, results in significantly higher intracellular ROS levels, compared to H_2O_2 alone. Strikingly, Cap1 is hyper-oxidised under such conditions and fails to accumulate in the nucleus and activate antioxidant encoding genes. As combinatorial oxidative and cationic stresses contribute to the potency of neutrophils in *C. albicans* killing, we are delineating the mechanisms underpinning Cap1 over-oxidation and inactivation. In addition, as combinatorial stress-mediated synergistic killing is observed in other fungi, the conservation of such stress-pathway interference mechanisms is being investigated.

Please note: Abstracts are published as received from the authors and are not subject to editing.

SU07/15

Ecdysone mediates maturation of the immune system of *Drosophila* embryo

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Activation of the innate immune system is crucial in both vertebrates and invertebrates to efficiently eliminate invading pathogens. This includes the induction of antimicrobial peptides (AMPs) and their subsequent secretion into the circulatory system; a phenomenon which has been highly characterised in *Drosophila* adult and larval models. Recent in vitro studies have suggested that the steroid hormone 20-hydroxyecdysone (20HE), a polyhydroxylated steroid hormone that plays key roles in reproduction, aging and development, may also play a regulatory role this process. Here we show that 20HE signaling in vivo is crucial to the development of a mature AMP response within the Drosophila embryo. Whilst embryos at Stage 15 of development were able to elicit a robust AMP response to bacterial stimulation, Stage 11 embryos exhibited a compromised immune response in terms of both AMP expression and survival after bacterial challenge. The AMP response of these early stage embryos can be rescued by treating with 20-hydroxyecdysone whilst disrupting 20HE signaling in Stage 15 embryos rendered them susceptible to infection and unable to initiate AMP induction. Therefore, 20HE signaling represents the mechanism by which maturation of immune signaling is mediated.

SU07/16

FCM assessment of the effects of simulated gastric conditions on spores of *Bacillus subtilis*

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The EU ban on the administration of antibiotics to animals as prophylactics, has caused an increased demand for ways to promote safe animal growth. *Bacillus subtilis* is commercially available as a probiotic to improve the health of animals; however their efficacy is not fully known. This may in part be due to the fact that spore behaviour in the gastrointestinal tract (GIT) is not completely understood. In this study, germination and inactivation of spores was examined in a variety of conditions that would simulate their passage through the stomach of pigs. Spore viability was analysed by plating, and the level of germination of the spores was assessed by DPA release measured at OD_{270}. In conjunction with these methods, flow cytometry (FCM) was also implemented to detect the different physiological states of *B. subtilis*. It was found that in a rich medium, after 30 minutes, 25% of spores were in the germinated stage and 38% had outgrown. The addition of 55mM HCl after 30 minutes of germination caused no further germination and above 80% killing of the germinated spores. The speed and accuracy of FCM gives clear advantages over traditional techniques to monitor changes in spore physiology.

SU07/17

The effect of novel antimicrobials on *Campylobacter* and the caecal microbiome of broiler chickens

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Thermotolerant campylobacters cause campylobacteriosis and are the leading cause of bacterial gastro-enteritis in the industrialised world. These bacteria thrive in the avian gut and the primary source of human infection is through the food chain via poultry meat, with horizontal transmission of the pathogen occurring after the bird is slaughtered.

Here we report on the use of the GRAS registered probiotic *Bacillus subtilis* incorporated into commercial feed and the organosulphur compound allicin, obtained from crushed garlic, and provided in drinking water, as alternative methods to reduce the load of thermotolerant *Campylobacter* spp. in Ross 308 broiler chickens. We highlight the use of traditional microbial techniques for culture and enumeration of *Campylobacter* spp. and comment on the use of 16s rRNA sequencing to determine changes in the caecal microbiome of broiler chickens fed these supplements.

SU07/18

Carbon utilisation impacts host–pathogen interactions during *Candida albicans* infection

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The human fungal pathogen *Candida albicans* transitions from a commensal lifestyle in the gut to a pathogenic lifestyle during systemic infection via passage into the bloodstream. Throughout this process, *C. albicans* resides in glucose-poor microenvironments, necessitating the utilisation of alternate carbon sources. Previously, our lab has demonstrated that growth *in vitro* on different, physiologically relevant carbon sources has implications for pathogenesis *in vivo*. For example, growth on lactate alters the composition of the *C. albicans* cell wall, modulates the induction of cytokines by mononuclear blood cells, and impacts stress resistance. Furthermore, pre-growth on alternate carbon sources impacts virulence in the systemic model of infection: lactate-grown cells are more virulent than glucose grown cells, and oleate-grown cells are less virulent. In this work, we examine the role of pre-adaptation to different carbon sources in proliferation and pathogenesis *in vivo*. We demonstrate that carbon source impacts interaction with immune cells, including macrophages and neutrophils, and alters the relative virulence of wild type *C. albicans* infections. Future work will examine the role of carbon source in tolerance of and adaptation to *in vivo* stress conditions and the impact of this adaptation on proliferation during systemic infection.

SU07/19

Associations between properties linked with persistence in a collection of *Staphylococcus aureus* strains isolated from bovine mastitis

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Staphylococcus aureus is recognised worldwide as a pathogen causing many serious diseases in humans and animals and is the most common etiological agent of clinical and subclinical bovine mastitis.

The aim of the present study was to investigate and correlate properties of *S. aureus* strains isolated from milk of cows suffering from mastitis, that may be associated with persistent mastitis: (i) expression of capsular antigens (CP5 or CP8) by specific ELISA; (ii) intracellular survival by invasion of MAC-T cells; and (iii) biofilm production by spectrophotometry analysis after growth in TSB_{glc}.

The results showed that (i) the proportion of strains expressing capsular antigen was higher in *cap8*- than in *cap5*-positive isolates; (ii) a correlation was observed between intracellular survival and both the capsular genotype and phenotype; and (iii) the biofilm production was associated with the capsular phenotype but not genotype. Therefore, isolates expressing the capsular antigen CP8 with low intracellular survival are probably better adapted to an extracellular niche and could be specifically associated with production of clinical mastitis. Conversely, isolates that do not express any capsular antigen (CP5 or CP8) with high intracellular survival are probably better adapted to an intracellular niche and could be specifically associated with production of subclinical mastitis and persistence of the infection.

In conclusion, capsular profile, biofilm production and intracellular survival analyses could be used as prognosis tests, to predict the persistence of the infection in the case of mastitis caused by *S. aureus*.

SU07/20

Direct dilution of cell aliquot for high temporal resolution sampling in bacterial surface charge measurement

WENFA NG

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Bacterial surface charge (SC) mediates important cell-environment and microbe-host interactions. Accurate and precise measurement of SC, by microelectrophoresis, requires removing metabolites adhered to the cell surface – where repeated washing by buffers is the standard approach. Nevertheless, the need for time-consuming centrifugation limits the temporal resolution of sampling for probing experimental dynamics. Herein, diluting cell aliquots with a buffer was investigated as a rapid and simple sample preparation technique, by examining the effects of dilution factor, type of cation, and buffer conductivity on *Escherichia coli*'s SC measurement. Results indicated that dilution factor was critical to accurate SC measurement since low signal-to-noise ratios in high or low cell concentration samples generated substantial error. Type of cation in buffer was also important since those with high surface affinity bind to the cell surface – resulting in under-estimations. Finally, although high conductivity buffers enabled greater removal of adsorbed metabolites; at extreme conductivity/concentration, both intrinsic ions and adsorbed metabolites were removed. Intermediate conductivity buffers also led to greater uncertainty in measured SC. Altogether, with low conductivity, deionised water reliably reproduced SC values obtained using the standard approach; however, since the ensemble of secreted metabolites is bacteria/medium specific, distinct optimal parameters exist for each system.

CMM
Clinical and medical microbiology

CMM/01

Charts plus chat required for effective administration of gentamicin

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Introduction In NHS Ayrshire and Arran, the first-line antibiotic regime for intra-abdominal sepsis has been changed to Amoxicillin, Metronidazole and Gentamicin, in order to reduce antibiotic resistance and Clostridium difficile infection. We aimed to audit the effectiveness of a dedicated Gentamicin prescription chart.

Setting Surgical wards in University Hospital Crosshouse.

Method Ward survey performed using a proforma from 03/09/2013 to 21/09/2013. The result was reflected to the medical and nursing staff. A repeat audit was performed from 18/02/2013 to 08/03/2013. Analysis was performed using SPSS.

Results There were 16 and 15 patients in cycle one and two respectively. Gentamicin prescription chart usage and calculation documentation was similar between cycles, (100% vs. 100%) and (94% vs. 87%, $p = 0.505$) respectively. Age, sex, height and weight documentation were comparable between cycles. After feedback to staff, Gentamicin administration start time documentation improved from 81% to 100% ($p = 0.078$). Gentamicin monitoring also improved from 56% to 87% ($p=0.062$). The rate of missed or late administration of Gentamicin dropped from 38% to 13% ($p=0.12$).

Conclusion Although a dedicated Gentamicin chart is useful, focused education to medical and nursing staff is the key to accurate and safe administration of Gentamicin.

CMM/02

The effect acidified nitrite has on clinical methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*, and human keratinocytes

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Nitric oxide (NO) is an integral part of various bodily functions including vasodilation of smooth muscle, neurotransmission, regulation of wound healing, and non specific immune responses to infection. There is a substantial level of experimental evidence that NO in various forms exhibits antimicrobial activity against a wide range of organisms including fungi, parasites, helminths, protozoa, yeasts, mycobacteria and bacteria. It is believed that sodium nitrite in an acidic environment results in the bactericidal effects seen against such pathogens. This study demonstrates the sensitivity of methicillin-resistant *Staphylococcus aureus* (MRSA) to clinically optimised formulations of sodium nitrite and citric acid. Several formulations have demonstrated bactericidal activity after 10 minutes exposure to various MRSA clinical isolates extracted from different skin wounds. The effects of these treatments were also observed in clinical methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates and the toxicity of the formulations were investigated against human keratinocytes to demonstrate the viability of the combination as a topical treatment for skin infections.

CMM/03

Characterising the human immune response to schistosomiasis

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Background: The role of monocytes in the innate immune response in infection models is established, but is unknown in schistosome exposed human populations. This novel study ascertains monocyte relationships with parasite specific cytokine and antibody levels in a Zimbabwean population endemically exposed to schistosomiasis.

Methods: PBMCs from 96 Zimbabweans (aged 5-54 years) in an area endemic for schistosomiasis infection were analysed via flow cytometry for the proportion of monocyte subsets, according to expression of CD14 and CD16 surface markers. Levels of systemic cytokines (IL-4, IL-5, IL-10 and IFNg) egg (anti-SEA) and worm (anti-SWAP) antibodies (IgA, IgE, IgG, IgM) were measured by ELISA. Association with monocyte subsets was investigated using ANOVA.

Results: CD14+CD16+ monocytes increased with increasing levels of Th2 cytokines IL-4, IL-5 and IL-10 ($p=0.042$, $p=0.022$, $p=0.020$ respectively) and exhibited infection level-dependent positive relationships with IgA, IgE and IgG ($p=0.011$, $p=0.003$, $p=0.034$ respectively). CD14++CD16+ were positively related with the protective antibody IgE SWAP ($p=0.010$, $\beta=0.906$)

Conclusions: These results suggest a relationship between CD14++CD16+ monocytes and systemic parasite-specific cytokines and antibodies in people exposed to schistosomiasis. Correlation between the monocytes with antibodies associated with protection and low infection levels suggests that this monocyte subset is also associated with protection against infection.

CMM/04

Withdrawn

CMM/05

Differential expression of the *Helicobacter pylori* vacuolating cytotoxin gene, *vacA*, in the human stomach

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The vacuolating cytotoxin (VacA) is one of the main virulence factors produced by *H. pylori*. Certain *vacA* genotypes are strongly associated with increased disease risk, but this association is not absolute. We hypothesised that the amount of VacA produced is also important and aimed to characterise *vacA* expression *in vivo* and its association with promoter region polymorphisms and disease.

Using RT-qPCR, we measured *vacA* mRNA levels in antrum and corpus biopsies isolated from 15 *H. pylori* positive patients. Sydney scoring was performed on histological sections and the *vacA* promoter region was sequenced from isolated strains.

We found that *vacA* transcript levels varied over a 19 fold range. For most strains *vacA* expression was higher in the corpus than the antrum (median 1.78 fold difference; $p=0.04$, Wilcoxon matched-paired signed rank test). Extensive variation existed within the *vacA* promoter region and several common polymorphisms within regulatory regions were identified. No significant correlation between promoter region diversity, *in vivo* *vacA* transcription level, inflammation score or disease status was found.

Increased *vacA* mRNA levels in the stomach corpus suggest a role for environmental factors regulating *vacA* transcription. We aim to investigate this further by assessing the effect of pH on *vacA* transcription *in vitro*.

CMM/06

Mutational analysis of loop 2 of the Hek invasin

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Neonatal meningitic Escherichia coli (NMEC) are known to be the principal cause of gram negative neonatal sepsis and meningitis. NMEC have a complex pathogenesis, which can be broken down into three key stages. The final two stages, which involve the development of bacteraemia, passage across the blood brain barrier and inflammation of the meninges have been extensively studied, but the mechanisms behind bacterial transcytosis from the intestine into the bloodstream have yet to be fully elucidated.

Previous work from this lab has shown that Hek, an outer membrane protein expressed in NMEC strain RS218, plays a role in adherence to and invasion of epithelial cells. The protein is purported to have four external loops, and of these four external loops, loop 2 has been identified as being essential for invasion. Bacteria expressing the Hek protein can also promote autoaggregation and cause haemagglutination.

In order to identify the essential residues in loop 2 required for cellular interactions, alanine-scanning mutagenesis was performed. The following charged amino acids were changed to alanine; R73, K75, D77, K79, D83, K84, D85, R93, D94, D95 and D97. Bacteria expressing mutant Hek proteins were assayed for haemagglutination, autoaggregation and invasion.

CMM/07

Expression of quorum sensing genes in the development of *Pseudomonas aeruginosa* biofilms during different states of growth

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Biofilm formation in *Pseudomonas aeruginosa* is thought to follow a developmental pattern initiated by adherence to a surface, followed by micro-colony formation and mature biofilm establishment. This process is influenced by cell-to-cell communication systems – quorum sensing (QS), (*las*/*rhl*) that regulate the expression of diverse exoproduct virulence determinants. Interrupting these pathways is currently being explored as a novel alternative to conventional antibiotics. An *in vitro* microplate-based model was developed to determine the expression intensities of eight QS-regulated genes, and the production of a QS exoproduct, pyocyanin, in PAO1 biofilms developed under static or dynamic conditions on medical grade stainless steel. Qualitative RT-PCR identified expression at key stages of biofilm development (1h, 6h, 24h, and 48h). Over time, biofilms composed of comparable cell densities transcribed and produced significantly less RNA and pyocyanin, respectively, when under dynamic conditions. Five of the genes investigated were predominantly expressed in 6h-old-static-biofilms, with varying abundance across the remaining time-points. It was determined, however, that expression of the *rhl* system genes differs in immature biofilms under these growth states. These findings highlight the stages of development at which QS inhibitors could be used to delay the establishment of *P. aeruginosa* biofilms, and to achieve maximal QS disruption.

CMM/08

Metatranscriptomics of colonic bacteria in inflammatory bowel diseases

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Crohn's disease and ulcerative colitis are inflammatory bowel diseases (IBD) characterised by chronic and relapsing inflammation of the gastro-intestinal tract. They cause lifelong suffering, as well as considerable drainage of health care resources. Although their etiology is still unclear there is a growing body of evidence for a significant microbial factor. In this study we focus on the global gene expression of these communities through mRNA sequencing. We collected colonic biopsies from inflamed and non-inflamed colonic mucosa in 19 IBD patients and using RNA-Seq with unprecedented depth we compared microbial metatranscriptomes in inflamed and non-inflamed colonic mucosa. This was done using 600Gb of Illumina HiSeq RNA-Seq technology (15Gb/sample). Bacterial reads were mapped against a reference database constructed from all sequenced bacterial and viral genomes from the gastrointestinal subset of the human microbiome project. We subsequently used DE-Seq to analyze the count data. From this we observed higher expression rates of E.coli in inflamed mucosa, as has previously been observed in CD patients. This analysis added a multitude of gene candidates that were significantly up/down regulated in inflamed tissue. Thus, our analysis revealed transcriptional differences for known microbial pathogens.

CMM/09

A structural and functional study on a cancer toxin from *Bacillus thuringiensis*

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Bacillus thuringiensis produces a range of toxins that include both the insecticidal Cry toxins and non-insecticidal, non-haemolytic, Parasporins. The latter target specific human cancer cell lines. One of these Parasporins (Cry41Aa-Parasporin 3) contains the five conserved sequence blocks found in many insecticidal toxins and is also believed to possess the same three domain fold. Cry41Aa is however predicted to have an extra loop in its domainII as well as an additional "ricin" domain at its C-terminus. To test whether these additional structural elements were responsible for the human cell activity of this toxin two deletion mutants were constructed. Deletion of the loop region resulted in an unstable protein that could not be further analysed. Deletion of the "ricin" domain resulted in a stable protein with a toxicity to HepG2 cells not significantly different to the non-modified toxin. To further investigate which regions of Cry41Aa may be responsible for specificity we mutated other loops in domainII that had previously been implicated in determining specificity in insecticidal Cry toxins. Alanine substitution mutants here abolished activity against HepG2 without otherwise affecting the toxin. This suggests that the nature of specificity determination may be the same with this Parasporin as with insecticidal toxins.

CMM/10

Mode of action of a human cancer cell active toxin from *Bacillus thuringiensis*

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In this study the cytocidal activity associated with a non-insecticidal and non-haemolytic protein toxin of *Bacillus thuringiensis* (*Bt*), known as Parasporin-3, was characterised. We investigated the effects of recombinant Parasporin-3 on the human hepatic cancer cell line HepG2 to elucidate its mode of action. The fact that some *Bt* toxins are able to kill mammalian cells may threaten the use of *Bt*-based pesticides in the future. Parasporin-3 shares structural homology with commercially used insecticidal toxins and is toxic to a narrow range of cell lines. At present, the cytotoxic effects of other Parasporins (1, 2 and 4) have been closely examined, and each toxin appears to have a different mode of action. Our results suggest that Parasporin-3, like its insecticidal homologues, is a pore-forming toxin that rapidly increases membrane permeability in the target cell. Significant uptake of fluorescent dye was observed in susceptible cells as little as 10 minutes post administration, suggesting rapid membrane damage. The activation of apoptosis effectors Caspase 3/7 was not observed within 8 hours. Microscopic observation revealed cellular and nuclear swelling induced within a few hours of treatment.

CMM/11

A novel virulence strategy for *Pseudomonas aeruginosa* mediated by an autotransporter with arginine-specific aminopeptidase activity

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The superbug *Pseudomonas aeruginosa* is responsible for a broad range of diseases including life-threatening infections, especially in patients with wounds, burns and cystic fibrosis. There are four proteins exhibiting the characteristic three domain structure of autotransporters in *P. aeruginosa*. Most of the autotransporters has been characterised as virulence factors. Here, we reveal that the PA0328 autotransporter is a cell-surface tethered and arginine-specific aminopeptidase. Hence, we have named PA0328 AaaA. We demonstrate that AaaA offers a fitness advantage in environments where the sole source of nitrogen is peptides with an amino terminal arginine. This could be vital for establishing an infection as the lack of AaaA led to attenuation in a murine chronic wound infection which correlated with lower levels of the cytokines TNFα, IL-1a, KC and COX-2. Moreover, we show evidence that aaaA could be regulated by genes linked with the complex quorum sensing system of *P. aeruginosa*. Structural modelling has identified the putative active site of AaaA, and mutants of AaaA with single amino acid changes created. We will show how these mutants are enabling us to fully define the active site of AaaA thereby facilitating the screening for inhibitors that could be exploited as potential therapeutic agents.

CMM/12

Induction of stringent response in *S. aureus* by mupirocin

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In most bacteria, nutritient deprivation induces the stringent response and bacteria start to synthesise (p)ppGpp, an alarmone that regulates transcriptional and translational control mechanisms which enables the cell to adapt to stress conditions. This adaption is processed through huge alterations in gene expression which may change the cell properties such as virulence and persistence.

Relatively little is known about the stringent response in S.aureus, in particular the effects of long term exposure to sub-inhibitory concentrations of mupirocin have not been studied. In this work, the production of (p)ppGpp after exposure to mupirocin was determined by HPLC for S.aureus strain 8325-4. (p)ppGpp concentration reached a maximum yield of 3.5 nmole/mg CDW after 1 h decreasing to1.19 after 24 h and dropped to 0 48 h after mupirocin treatment. Moreover, the stress response effect on gene expression for TST and RNAIII in S.aureus has been observed by RT-PCR.

A detailed study of the stringent response and its effect on virulence gene expression in S.aureus will enhance our understanding. Observing (p)ppGpp production in S.aureus might expand the knowledge of its adaption in difficult environment which may lead to a new approach to fight the organism.

CMM/13

High prevalence of antibiotic resistant *Mycoplasma genitalium*: the need for routine testing and the inadequacy of current treatment options

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Empirical antibiotic therapy for non-gonococcal urethritis (NGU) prioritises *Chlamydia trachomatis* over *Mycoplasma genitalium*. However, the latter is not routinely tested for and is not effectively treated by preferred chlamydia treatment. Prevalence of *M.genitalium* and associated genotypic markers of macrolide and fluoroquinolone resistance, among men symptomatic of urethritis, were investigated. Population diversity of *M.genitalium* was determined to infer whether findings were applicable beyond our setting. Methods: *M.genitalium* was detected using nucleic acid amplification methods, and DNA sequencing was used to detect genotypic resistance markers of macrolide and fluoroquinolone antibiotics. An established typing method was used to assess population diversity. Findings: Among 217 men, *M.genitalium* prevalence was 16.7% (95%CI: 9.5-24.0) and *C.trachomatis* 14.7% (95%CI: 7.8-21.6) in NGU cases. 9/22 (41%; 95%CI:20%-62%) patients with M.genitalium were infected with strains possessing macrolide resistance and one patient with a fluoroquinolone resistant strain. Typing assigned *M.genitalium* strains to two major clusters, broadly distributed among previously typed international strains. Genotypic macrolide resistance was spread within these two clusters. Interpretation: *M.genitalium* is a frequent cause of NGU in this population with high rates of macrolide resistance. Guidelines for testing and treatment of NGU should be modified to reduce treatment failure and the selection of further resistance.

CMM/14

Association of selected virulence genes with *mecA* gene on genome of *Staphylococcus aureus* strains from Nigeria

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Background. The pathogenicity of Staphylococcus aureus depends on the presence of virulence factors. This study was aimed at using PCR to determine the prevalence of mecA, and selected virulence genes on the genome of clinical isolates of S. aureus from Nigeria.

Methods. A total of 156 S. aureus strains isolated from clinical samples submitted from Nigeria were used. Polymerase chain reaction (PCR) technique was used to screen for the presence of mecA, and some selected virulence genes. PCR discrimination of MRSA isolates into CA-MRSA and HA-MRSA using MW and SCCMec typing schemes.

Results. The prevalence of mecA gene was reported to be 42.3%, hla (55.1%), ica (42.3%), PVL (34.6%), fnbA (8.3%), bbp (4.5%), and eta (3.8%). Prevalence of pvl gene was found to be 53.3% on mecA- when compared to 9.1% seen in mecA+ strains. MIC results for mecA+ strains was >256 µg/ml while the MIC for mecA- strains was ≤4 µg/ml of methicillin. The result of SCCmec typing revealed 24 (36.4%) SCCmec II, 4 (6.1%) SCCmec III, 10 (15.2%) SCCmec IV, and 28 (42.4%) SCCmec V of the total 66 mecA+ S. aureus strains.

Conclusion. Association was found between virulence genes and mecA- strains ($X2 = 13.7$; $P < 0.01$).

CMM/15

An optimised MALDI-TOF-MS protocol for differentiation of clinical staphylococcal isolates including MRSA

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Limited success has been reported for matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) differentiation of staphylococci, including MRSA strains, using non- or partially-optimised procedures. In the present research, a MALDI-TOF-MS protocol was developed through systematically optimisation and applied to differentiation of three sets of clinical staphylococcal isolates: *S. aureus* (SA; 36 isolates); MRSA (30 isolates); other *Staphylococcus* species (NSA; 15 species, 48 isolates). Combinations of chemical pre-treatment of cells on target plates (solvents, reductants, detergents, acids) were introduced to enhance spectral richness. Other parameters for optimisation were: growth medium; matrix chemical; target plate inoculation method; and peak picking/processing criteria.

The optimised protocol involved: growth on Muller Hinton agar; single colony transfer to target plate; application of formic acid:isoproponal:H_2O (13:33:50) as chemical pre-treatment; use of α-cyano-4-hydroxycinnamic acid as matrix chemical in ACN:H_2O (2:1) and 2% trifluroacetic acid; and discrimination by species specific peaks rather than by total peaks. Inclusion of the chemical pre-treatment step, in particular, increased the number of species specific peaks detected over the 2,000-7,000 *m/z* range; typically 34 *S. aureus* specific peaks within a total of 754 detected. MRSA isolates were differentiated from NSA isolates with 100% accuracy, and from other SA with 77% accuracy.

CMM/16

Development of IgG antibodies to *Clostridium difficile* toxins using a plant based expression platform

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Clostridium difficile is an anaerobic toxigenic bacterium that is a major cause of hospital acquired diarrhoea and pseudomembranous colitis.

The current treatment approach for *Clostridium difficile* infection is antibiotics, but the emergence of more resistant strains has warranted the need for the development of novel therapeutic approaches, specifically non-antibiotic based ones. Recent work has found that toxin specific antibodies have the ability to bind to and neutralise the toxins produced in vivo and control the severity of *Clostridium difficile* associated disease (CDAD).

We have collected a range of anti-toxin antibody sequences previously expressed using traditional methods and modified them for a plant based system.

Humanised IgG antibodies against TcdA and TcdB were expressed and purified following transient infiltration of *N. benthamiana*. ELISA and cell culture assays were used to demonstrate the specific binding and neutralisation abilities of these antibodies. Antibodies were found to have varying binding and neutralising effects on TcdA and TcdB.

In conclusion, the development of alternative therapeutic options for the treatment of infectious diseases is of great importance; plant based expression platforms are an emerging field in the development of protein pharmaceuticals and offer several advantages including the potential for large scale production with relatively low tech equipment.

CMM/17

Regulatory control of integron-mediated antibacterial resistance

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Resistance to last-line carbapenem antibiotics is a global health concern, with mobile integrons identified as a major route of carbapenemase dissemination. Integrons are naturally occurring gene traps which recruit and excise gene cassettes under the control of the integron integrase, in response to environmental triggers.

Our study aims to examine the regulatory networks involved in integron activities in clinically relevant pathogens; particularly, dissection of the response of integrons to SOS induction, and the identification of novel regulators.

We have developed a dual reporter of integron promoter activities from a clinically-derived mobile integron, with which we are studying integron regulation in the nosocomial pathogen *Pseudomonas aeruginosa* and the model bacterial species *Escherichia coli*. We observed SOS regulation of the integrase promoter in both bacterial species to differing degrees, through comparison of constructs with intact and mutated SOS boxes. We are now undertaking a high-throughput screen of a *P. aeruginosa* transposon library for mutants displaying aberrant promoter activities.

This study is the first to demonstrate that regulation of integron promoter activities differ significantly between model, and clinically-relevant, bacterial species, and may therefore begin to explain the predominance of new gene variants in some species.

CMM/18

Enhancing *Staphylococcus* biofilm formation on biomedical polymer material

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Bacteria exist in two basic states, planktonic cells or sessile cells. Studies show that adherent bacteria growing in consortia are known as biofilms and are present in virtually all natural and pathogenic ecosystems. These biofilms are defined as a community of micro-organisms irreversibly attached to a surface, producing extracellular polymeric substances, also known as E.P.S. Most importantly, the biofilm is characterised by its resistance to biocides, antibiotic chemotherapy, and clearance by humoral or cellular host defence mechanisms.

This work will focus on establishing biofilm formation for selected bacterial species and optimising a 96-well microtitre plate method for screening anti-biofilm forming activity. The aim of this work is to optimise the MicroTitre Plate-based assay. This system is used as it allows researchers to easily vary multiple parameters including composition of media, incubation temperatures, humidity, presence or absence of shear stress, and oxygen concentrations.

Model systems need to be developed and used to study biofilm processes and formation on various indwelling devices. The aim of this work is to provide an assay that will optimise biofilm formation so that it can provide a robust biofilm in which novel antimicrobials can be tested on.

ENV
Environmental microbiology

ENV/01

Prevalence of mobile genetic elements among bacteria isolated from petroleum hydrocarbon polluted and unpolluted ecosystems in the Niger Delta region of Nigeria

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This study was undertaken to investigate the prevalence of mobile genetic elements among multiple antibiotic and heavy-metal resistant bacteria from petroleum hydrocarbon polluted and unpolluted ecosystems in the Niger Delta Region of Nigeria. Three hundred and forty-five (345) bacterial isolates, mostly Gram-negative, were screened to determine their heavy metal tolerance and antibiotics resistance profiles using standard microbiological procedures. Of the 345 isolates, 76 (37 from polluted and 39 from unpolluted ecosystems) exhibiting resistance to multiple antibiotics and tolerance to several heavy metals were screened for integrons by polymerase chain reaction based technique while plasmids were extracted following alkaline lysis and analyzed electrophorectically. Plasmids and class 1 integrons were present in 51.4% and 86.5% of the isolates from polluted ecosystems in comparison with 30.8% and 46.2% of isolates from unpolluted ecosystems, respectively. Differences between isolates from polluted and unpolluted sites in terms of prevalence of these genetic elements were statistically significant ($P<0.05$). Particularly the presence of class 1 integrons was significantly ($p<0.0003$) higher in isolates from polluted sites. Our findings show that petroleum hydrocarbon pollution selects for mobile genetic elements, possibly contributing to the dissemination of antibiotic resistance genes, raising public health concern.

ENV/02

Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in the final effluent of a wastewater treatment plant in the Eastern Cape, South Africa

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The prevalence of *Escherichia coli* O157:H7 in the final effluent of wastewater treatment plants in Eastern Cape is poorly understudy. *Escherichia coli* O157:H7 presence was determined using standard methods by a culture based method using chromogenic *E.coli* O157:H7 selective media while molecular based techniques was used for confirmation of the isolates. Sampling of final effluent for isolation of *E. coli* O157:H7 was done up to 9 months. Plate counts ranged between 0 log/ml–3.7 log/ml. A total of 335 were isolated. The overall prevalence was 33% for the 9 months sampled for which detection was positive using the selective media. The identification of poor final effluent wastewater management practices associated with colonisation

of by *E. coli* O157:H7 suggests the possibility of human exposure may be of a public health concern. Appropriate management of wastewater is necessary so that contamination of the environment and food by the organism can be prevented.

ENV/03

Mutating *E. coli* with the *arxA* gene: a novel, practical solution to the global arsenic water problem

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Arsenic, a poison found in water, exists in the environment in mainly two states: arsenite (carcinogenic and water-soluble) and arsenate (easily removed from water), with arsenite being most predominant. Extremophilic bacteria such as MLHE-1 have a gene called *arxA*, which enables them to change toxic arsenite into less-toxic arsenate. In this study, a new bacteria strain was created by inserting *arxA* from MLHE-1 into a nonpathogenic, common bacteria, E. coli strain K-12. This mutated E. coli strain is an ideal choice for practical water bioremediation.

arxA was extracted from MLHE-1, and the gene was amplified using PCR. The gene was put on a plasmid using restriction digests and ligation; this plasmid was used to transform E. coli by heat shock. After confirming that the *arxA* gene was in the E. coli, the newly-mutated bacteria was tested for arsenite-to-arsenate conversion. Two samples of bacteria (normal E. coli and mutated E. coli) were subjected to 25 ppm arsenite media and analyzed over a set time interval. Arsenite and arsenate amounts in both media were compared in both samples at set time points. The mutated E. coli with the *arxA* gene (unlike the normal E. coli) successfully converted arsenite to arsenate.

ENV/04

Poly-gamma-glutamic acid (γ-PGA) – a promising biosorbent for removal of heavy metals

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Poly-gamma-glutamic acid (γ-PGA), an unusual natural anionic biopolymer composed of D- and/or L-glutamic acid units polymerised through amide linkages between α-amino acid and γ-carboxylic acid groups, was synthesised by three bacterial strains – *Bacillus subtilis* (natto), *Bacillus licheniformis* 9945a and *Bacillus licheniformis* 9945. Three culture media – one containing glycerol, citric acid and L-glutamic acid as carbon sources, another, having citric acid, sucrose and L-glutamic acid as carbon sources and the third one with sucrose and L-glutamic acid as its sources of carbon were used in this study. Each strain produced γ-PGA extracellularly when grown aerobically in one or all three media. The biopolymers produced were identified as γ-PGA by Fourier transform infrared spectroscopy (FTIR). The effects of different fermentation temperatures (37°C and 50°C) and media on bacterial growth, production and molecular weight of γ-PGA were investigated. The metal binding affinity of γ-PGA was also studied and it was found that it binds heavy metals. The optimal γ-PGA yield of 11.45g/l was obtained when *Bacillus subtilis* (natto) was grown aerobically at 37°C for 96 hours in a culture medium containing having citric acid, sucrose and L-glutamic acid as carbon sources.

ENV/05

Bioremediation of crude oil-contaminated soil using bacteria and zeolite

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Bioremediation is an important, cost effective and environmental friendly method used to clean up the soil and the environment from petroleum hydrocarbon contaminants utilising indigenous or selected microbial flora. The bioremediation of crude oil artificially contaminated soil by a mixed culture of two hydrocarbon-degrading bacteria, Rhodococcus spp and Pseudomonas spp, was investigated. These bacterial strains were selected based on criteria that they were able to utilise hydrocarbons as the sole source of carbon and energy and were able to show significant growth in crude oil. The influences of a zeolite (clinoptilolite) and inorganic nutrient additions on the biodegradation of crude oil in soils were investigated. Soil amendment experiments at 30°C for a period of 30 days showed a more rapid and greater extent of apparent oil removal with the addition of both bacterial consortium and clinoptilolite. There was 79% oil removal by the bacterial consortium in the soil amended with clinoptilolite as compared to 67% in the case of the amended soils without clinoptilolite. Although, the addition of both bacterial consortium and clinoptilolite enhanced the removal of the crude oil, however the effect of clinoptilolite may be one of abiotic removal.

FB
Fermentation and bioprocessing (industry)

FB/01

Purification and characterisation of novel recombinant β-glucosidases from *Aspergillus*

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An initial bioinformatics analysis of the genome databases for β–glucosidases for *Aspergillus* has collated the variation in this enzyme class using keyword searches and Blast. Selection of novel candidates for direct gene synthesis for cloning and expression is based on size (short genes), pI (ExPASy – Tools) and novelty. Several *Aspergillus* strains have been screened using a rapid plate assay based on Congo Red. Selection of candidate strain was based on temperature profile, pH range and carbon source degradation. Potential bacterial donors of cellulose degrading enzymes have also been explored for their expression in fungal hosts. *Pichia pastoris* systems will be used for enhanced expression of *Aspergillus* proteins employing fusion PCR of the target gene with an inducible promoter. At the end of this work we hope to generate new β-glucosidases, cloned and analyzed for industrial use to produce biofuel (renewable energies).

FB/02

Investigation of the control of antibiotic production and sporulation in *Streptomyces coelicolor*: the role of specific small non-coding RNAs

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RNA-binding chaperone proteins in *Streptomyces coelicolor* A3 (2) MT1110 are investigated in this experiment. The aim is to discover chaperone proteins involved the post-transcriptional regulation of gene expression in the biotechnologically vital streptomycetes bacteria. Depending on experiments confirming the expression of many small non-coding RNAs in *S. coelicolor* A3 (2), scr3558-1 RNA was chosen for this experiment to find what chaperone proteins bind to these RNAs *in vitro*.

scr3558-1 gene was identified and cloned in pUC18 plasmid. T7 promoter was cloned upstream to *scr3558* and tobramycin aptamer downstream of the gene. The clone was PCR amplified and *in vitro* transcribed. scr3558-1 RNA was ligated to

tobramycin-coupled Sepharose beads. At this stage, *S. coelicolor* cell extracts taken from several points during the exponential and stationary phases were added to the RNA-Sepharose complex. RNA served as a bait to collect RNA-binding proteins. Consequently, RNA was eluted using tobramycin-containing buffer. Protein content of the elution was analysed using bioanalyzer. Mass spectrometer was then used to identify the protein amino acid sequence. The experiment is currently at the bioanalyzer stage and a 45kDa protein was identified in several replicates of the experiment. Mass spectrometer results will be shown on the poster.

FB/03

Identification of yeasts and fermentation volatiles from palm wine obtained in Imo State, Nigeria

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The local palm wine drink used in many traditional ceremonies in Nigeria was sourced from 6 different sites in Imo State region and analysed to determine the yeast species and products of their fermentation. Species and sub-species identification carried out using a combination of PCR amplification of ITS1-5.8 S rDNA-ITS2 regions, RFLP analysis and sequencing of D1/D2 domain of the nuclear 26S rDNA of yeasts isolated revealed that *Saccharomyces cerevisiae* was the dominant yeast (70% of the 42 strains analysed). Other yeasts isolated were *Pichia kudriavzevii, Candida tropicalis* and *C. ethanolica*. Three main volatiles that play a role in the aroma of the palm wine namely, ethanol, acetic acid and ethyl acetate were detected using atmospheric pressure chemical ionisation (APCI) and GC-MS. The ethanol content of the palm wine was estimated using HPLC and was found to be 4% after storage at 20 °C for 4 weeks. The detection of styrene, a monomer and precursor of polystyrene in all samples indicated that yeast fermentation of palm wine results in corrosion of plastic bottles used for its storage. This could be a health hazard since plastic bottles are widely used for the storage of palm wine in Nigeria.

GM
General microbiology

GM/01

Colourless agar for enhanced colour contrast between colonies and solid medium

WENFA NG

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Agar medium is useful for preliminary screening of microbial diversity by enabling spatially-separated concurrent cultivation of different microbes. Lack of colour contrast between colonies and agar, however, hampers colony identification by automated image analysis. Since microbes secrete pigments of myriad hues, this research aims to develop a colourless agar – which when placed on coloured paper of suitable hue – enhances the colour contrast between agar and colonies of any colour. Nevertheless, the concept is confounded by formation of coloured compounds between medium components during autoclave sterilisation – which, in this study, was prevented by dissolving glucose and NH_4Cl in two separate solutions containing other medium components, that upon mixing yielded a colourless agar – even after adding yeast extract (max: 1 g/L) for providing essential vitamins. Culture experiments revealed good growth of Escherichia coli, Pseudomonas fluorescens, Pseudomonas aeruginosa, and Bacillus subtilis with cell density positively correlated with yeast extract concentration. Additionally, identical viable cell concentration and colonies of similar morphologies were observed on both the colourless and LB agar; thus, suggesting that no inhibitory compounds were formed during agar preparation. Collectively, using common buffer, salts and nutrients, colourless agar was prepared by segregating chromogenic medium components during heat sterilisation.

GM/02

A microbiological assessment scheme (MAS) to evaluate the feed safety management system (FeSMS) in a category 3 fat melting establishment

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The performance of a FeSMS in a Category 3 beef fat melting establishment that manufactures greaves for pet food production was measured using a MAS. Over a 10-month-period 685 samples taken at 7 critical sampling locations (raw materials, greaves post heat treatment, post centrifugation, post packaging, the environment, personnel and water) were analysed for 8 microbial parameters (Total Viable Counts, *Enterobacteriaceae, Salmonella, Listeria* spp, faecal *enterococci, Escherichia coli, Clostridium perfringens*, coliforms). Findings were benchmarked against legal, industry and best practice norms. With reference to Regulation (EC) No. 2073/2005, all raw fat samples acheived the same acceptable criteria as the beef carcases from which it was harvested. *Salmonella* was not detected in final product samples, *Enterobacteriaceae* were isolated at levels of 15cfu/g and 165cfu/g samples. All water samples were compliant. Four environmental samples had TVC greater than 10cfu/cm^2 with *Listeria innocua* isolated from one. Two personnel swabs had TVC above the recommended 100cfu/cm^2. In conclusion, safe petfood is currently being produced and the MAS is an effective tool to assess FeSMS performance, however, post cooking contamination from the environment and personnel remain a risk. MAS provides the establishment with key information on where to prioritise resources to improve product safety.

GM/03

Comparative antimicrobial activities of different species of *Acalypha* on selected clinical isolates in Oauthc, Ile-Ife

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Antimicrobial activities of leaf extract of some species of Acalypha were investigated for medicinal purposes against Staphylococcus aureus, Escherichia coli, Psudomonas aeruginosa, Proteus mirabilis, Alpha heamlytic Streptococcus, Klebsiella pneumonia,Salmonella typhi, and Candida albicans using agar diffusion method. The extracts of Acalypha wilkesiana showed the greatest antimicrobial activity against Gram-positive, Gram-negetive bacteria and the fungus tested. Acalypha hispida burm showed antimicrobial activity against Gram-positive and Gram-negative bacteria while Acalypha ciliata was only effective against Gram-negative bacteria. Results from this study confirmed the broad-spectrum activities of some spicies of Acalypha plant which traditional medicine practitioners use in treating various ailments including fungi infections of the skin. The Acalypha extracts compared well with the commercial antibiotics tested against the isolstes.

GM/04

Investigating the role of rhomboid proteases in the development of *Dictyostelium discoideum*

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The rhomboid intramembrane proteases are conserved across all kingdoms of life and show notable structural similarity, although their functions within individual organisms remain largely unknown. The membrane location of rhomboids means they are ideally placed for roles in signaling and proteolytic activation events. We are investigating rhomboid function in Dictyostelium discoideum, a soil amoeba that is highly motile, capable of unicellular vegetative growth and multicellular development when exposed to starvation conditions.

Bioinformatics tools were used to identify four potential rhomboids encoded within the Dictyostelium genome that contain the residues necessary for proteolytic activity. To identify their functions, 3 null mutants rhmA$^-$, rhmB$^-$ and rhmC$^-$ have been constructed, and their growth and development observed.

Preliminary data demonstrates reduced growth rate in rhmA$^-$ and rhmB$^-$, both devoid of transcript known to be amplified via RNASeq. RhmA$^-$ responds atypically to cAMP and folic acid gradients, especially when undergoing prolonged starvation suggesting that the protein could function within the signalling pathway controlling development Dictyostelium in low-nutrient environments. RhmB- shows a characteristic developmental phenotype; smaller spore size with decreased viability. RhmC$^-$ does not have an identifiable phenotype in assays to date although transcription peaks at during the slug formation phase.

GM/05

Diversity of halophilic fungi from mangroves

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Halophilic and highly salt tolerant fungal genera from mangroves of Goa were isolated on high salt media. The mangroves are situated at the intertidal zone, which is the area of the shore and seabed that is exposed to air at low tide and submerged at high tide. These fungi belonged mainly to the genera Aspergillus and Penicillium, with some of these from the genera Cladosporium, Eurotium and Hortaea. All the isolates could grow at high sodium chloride concentrations up to 25%. There were significant differences in growth of each isolate at varied salt concentrations. Some of these showed a slow growth, while some had a faster growth pattern on the salt media. All were moderate halophiles, euryhaline in nature, with a wide range of salt tolerance.

GM/06

Investigating the role of the non-integrin laminin receptor in the pathogenesis of bacterial meningitis

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Despite major advances in medical treatment, bacterial meningitis continues to be a serious infectious disease. Some bacterial pathogens such as *Neisseria meningitis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* share common mechanisms to cross the blood brain barrier (BBB) and cause meningitis. Recently, it has been shown that a non-integrin cell surface lamnin receptor (LamR) is targeted by outer membrane proteins of these bacteria. This project aims to investigate the role of LamR in the pathogenesis of bacterial meningitis. To address this question, deletion and substitution mutations have been introduced into a LamR-expressing construct for expression LamR derivatives in mammalian cells. Wild type (WT) and mutant recombinant LamR were expressed in a human embryonic kidney cells (HEK 293T) and purified using an ÄKTAprime plus liquid chromatography system. The ability of these molecules to bind *N. meningitis*, *H. influenzae* and *S. pneumonia*, as well as their surface LamR-binding ligands, will be investigated; the results of these binding assays will provide a more detailed understanding of the role of LamR in the pathogenesis of meningitis.

GM/07

Molecular characterisation of a putative drug efflux pump MSMEG_2991 (efflux pump permease) of *Mycobacterium smegmatis*

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An important reason for the Mycobacterial drug resistance is the drug efflux *via* cell-membrane localised transport proteins known as efflux pumps proteins. Though many of them are reported in Mycobacteria, a large number of efflux pump proteins needs evaluation. In the present study, we attempt to characterise a putative efflux pump MSMEG_2991 of *Mycobacterium smegmatis*. MSMEG_2991 gene was cloned and overexpressed in *E. coli* 2443 strain that resulted increase in drug resistance about 16 fold for fluoroquinolones. An active antibiotic efflux by MSMEG_2991 was revealed by assessing the accumulation of *ciprofloxacin* in the absence and *presence* of efflux pump inhibitor, carbonyl chloride m-chloro phenyl hydrazone (CCCP). To check the protein *in vitro* MSMEG_2991 protein was purified in denatured state using Ni-NTA affinity chromatography and *in-silico* analysis of the protein suggested that it consisted of 12 transmembrane helices composed of mainly alpha-helices (α-80.56%, β sheet 0.47% and loop 18.97%). In a nutshell, MSMEG_2991 may possibly be designated as a fluoroquinolone efflux protein though such a hypothesis awaits further establishment.

GM/08

Studies on the effect of silicon compounds on microbial growth

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Silicon is the second most abundant element (after oxygen) on Earth, making up 27.7 % of the crust by mass, and is the eighth most abundant element in the universe. Silicon compounds, which are efficient at adsorbing gases and volatiles, remove combined carbon and nitrogen from the atmosphere, and these may then act as nutrient source for bacterial growth (Soomor, 2000). With the exception of diatoms, which contain silicon as a constituent part of their cell walls, a few studies have shown that fungi and bacteria can solubilise insoluble silicon compounds, it is likely that there exist important interactions between this element, bacteria and fungi (Wainwright. *et al.* 1997).

The present study with various bacteria species has indicated that bacteria dissolves insoluble inorganic silicon, which causes the release and accumulation of silicon, while several types of silicon compounds were examined to assess the effect of these compounds on Aurobasidium pulluans growth under normal and oligotrophic conditions. This proposes that silicic acid stimulates the growth of A.pulluans under normal conditions, whereas other compounds had an inhibitory effect on fungal growth oligotrophically.

In addition, the effect of silicic acid on the respiration rates of B.subtilis with HgCl2 have been studied.

GM/09

The GEF Sec2 binds its own mRNA specifically during the hyphal development of *Candida albicans*

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During hyphal growth, delivery of secretory vesicles to the hyphal tip is mediated by the RabGTPase Sec4 activated by its GEF Sec2. Sec2 localises to the Spitzenkörper and this localisation depends on phosphorylation by Cdk-Hgc1 and is essential for hyphal formation (Bishop, A. *et al*.. EMBO J. 2010 29:2930-42). Preliminary RNA-immunoprecipitation experiments suggested that Sec2 bound RNA during hyphal but not yeast growth. A Microarray experiment revealed the surprising result that Sec2 bound only its own mRNA during hyphal growth, a result confirmed by qRT-PCR analysis. We previously showed that a truncated Sec2 1-583 protein was unable to support hyphal growth whereas the Sec2 1-625 protein could support normal growth. Interestingly, Sec 1-583 protein was unable to bind to its mRNA, suggesting that the C-terminal 581-751 domain is required for mRNA binding and that the 581-625 region may be core. Significantly this region contains the key 584 residue which is phosphorylated. Sec2 binding to its own mRNA may be part of a mechanism ensuring that the Sec2 protein is concentrated in the Spitzenkörper that is a considerable distance from the nucleus where *SEC2* is transcribed.

GM/10

Transcriptomic changes in *Escherichia coli* associated with growth rate, and studying the gene regulator SlyA

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The gram-negative bacteria *Escherichia coli*, like all living organisms, must adapt to external pressures of the environment in which it grows. These adaptations are largely elicited by gene transcription regulators. Surprisingly little work has been done on what transcriptomic changes are coupled to a change in growth rate in *E. coli*. By growing the *E. coli* strain MG1655 in glucose-limited chemostat cultures it has been possible to define the growth rate by controlling the rate of dilution with fresh medium. This has allowed collection of RNA samples that have then been analyzed using microarrays to identify global transcriptional changes attributed to changes in growth rate.

Microarray analyses are also being carried out to identify genes that are controlled by the transcription regulator SlyA in *E. coli*, a homologue of a regulator attributed to control of a number of virulence genes in Salmonella typhimurium and yet only currently confirmed to control a single gene, *hlyE*, in *E. coli*.

GM/11

Transcriptomic and biochemical changes in *Escherichia coli* during a shift from fermentation to trimethylamine-*N*-oxide (TMAO) respiration

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Escherichia coli is a Gram-negative, metabolically versatile facultative anaerobe, which is able to utilise three different modes of metabolism for energy generation and growth. These metabolic modes include fermentation, anaerobic respiration and aerobic respiration. Trimethylamine-*N*-oxide (TMAO) is used by *E. coli* as an alternative terminal electron acceptor during anaerobic respiration. An osmolyte in marine organisms and a common metabolite, TMAO is reduced to trimethylamine (TMA) by *E. coli* during anaerobic respiration. Anaerobic TMAO respiration is more energetically efficient than fermentation, but less efficient than aerobic respiration.

Although both the regulation and operation of the *E. coli* TMAO respiratory chain (TorCAD) have been studied in detail, there is no understanding of the adaptive processes that occur during the transition from fermentative to TMAO respiratory growth. In this study, carefully controlled glucose-limited chemostat cultures have been used to study the adaptive processes in *E. coli* K-12 in response to TMAO using transcriptomics and metabolite profiling.

GM/12

Identification of toxin-antitoxin systems of PAO1 and study of their role in bacterial persistence

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Type II Toxin–Antitoxin (TA) protein pairs are encoded by adjacent, co-transcribed genes. Toxins mostly act as mRNases, decreasing the global translation rate in bacterial cells. Their activity is modulated by antitoxins that counteract the inhibitory effect of their cognate toxins by direct protein-protein interaction. Toxins are far more stable than their relative antitoxins. Indeed, the latter are rapidly degraded by proteases under unfavourable conditions. *E. coli* TA systems, along with the Lon protease, have been shown to be involved in the modulation of persistence upon treatment with high doses of antibiotics. Persistence is defined as a multidrug tolerance and it is not related to antibiotic resistance since it does not involve genetic changes. The Lon protease has been reported to activate TA loci-encoded mRNases in *E. coli* by catalysing the degradation of their cognate antitoxins. Consistently, Lon plays a role in the regulation of persister cell formation in this bacterium. Interestingly, TA systems are widely conserved amongst bacteria, including major pathogens. The aim of the present work is to study the yet uncharacterised TA systems of the opportunistic human pathogen *Pseudomonas aeruginosa*, focusing in particular on the possible involvement of these proteins in the regulation of persistence.

GM/13

Developing an *in vitro* model of infection of human airway epithelial cells with *Staphylococcus aureus* to investigate the effects of hyperglycaemia

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Background The luminal surface of airway epithelium is covered by a thin layer of airway surface liquid that is sterile despite being constantly challenged by inhaled pathogens. *Staphylococcus aureus* is a highly virulent bacterium, and its ability to adapt to the milieu of the respiratory tract has facilitated its emergence as a major cause of pneumonia in critically ill patients. Hyperglycaemia is a predisposing factor for staphylococcal lung infection.

Aims To establish an in vitro model of infection with *Staphylococcus aureus* and H441 airway epithelial cells to test the hypothesis that treating H441 cells with increased glucose concentration, prior to co-culture with the microbe, leads to enhanced staphylococcal binding.

Methods Using immunohistochemistry, staphylococci were localised in co-culture with H441 airway epithelial cells. H441

cells were cultured in media containing 10mM, 20mM and 40mM glucose and then infected with *S. aureus*. Bacterial counts were estimated by three blinded observers.

Results Mean staphylococcal count on H441 cells increased with higher glucose concentration. Statistical significance was observed when comparing bacterial count between cells cultured in 10mM and 40mM glucose ($P = 0.0483$).

Conclusions This preliminary study has demonstrated that exposing airway epithelial cells to higher glucose concentration facilitates staphylococcal binding to the cells.

GM/14

Phosphorylation of FKH2 is required for hyphal specific gene expression in *Candida albicans*

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Candida albicans Fork-head transcription factor, *FKH*2, has previously been shown to be important for both yeast and true hyphal morphogenesis; a trait believed essential for pathogenesis. We therefore sought to investigate the possible role of phosphorylation in the regulation of this influential transcription factor. Fkh2 exhibits a different pattern of phosphorylation during yeast and hyphal growth; undergoing cell cycle dependent phosphorylation during yeast growth, corresponding to periods of small bud growth; whereas in hyphae, phosphorylation occurs only during germ tube initiation, before formation of the septin ring. Fkh2 is a likely Cyclin Dependent Kinase (CDK) target, which is supported by phospho-peptide mapping data of Fkh2 during early hyphal growth. Mutation of the Serine or Threonine in the CDK consensus sites with a non-phosphorylatable alanine causes loss of the early hyphal phosphorylation, and results in morphological abnormalities. Initial microarray analyses of this mutant show a decrease in the expression of a number of canonical hyphal specific genes such as *HGC*1, *SAP*4-6 and *ECE*1. Thus cell cycle-independent phosphorylation of Fkh2 is a previously unsuspected requirement for normal hyphal development.

GM/15

Cell wall maturation/turnover-regulation mechanisms in *Bacillus subtilis*

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The bacterial cell wall is a dynamic structure that undergoes constant synthesis and degradation (turnover) during growth. The biosynthetic processes are wall studied, but the degradation and recycling aspects have received little attention and it has been suggested that old cell wall material is lost to the environment as new wall is synthesised and cell enlargement occurs. D-alanine has a central role in bacterial cell wall biosynthesis, particularly in peptidoglycan cross-linking in all bacteria, but also is a component of other wall polymers (Teichoic acids) in Gram-positive bacteria. After incorporation of new precursors into peptidoglycan, the terminal uncross-linked D-alanine residues are cleaved by DD-carboxypeptidase (DacA). Also, the D-alanine esters, which are attached to teichoic acids by the proteins of *dlt operon*, might be released by base-catalysed hydrolysis. This study investigates the fate of D-alanine and its uptake system to understand cell wall turnover and characterise specific recycling pathways for old cell wall components in *Bacillus subtilis* as a Gram-positive model.

GM/16

Structural and adhesive properties of AgI/II polypeptides in pathogenic streptococci

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Streptococcal antigen I/II (AgI/II) polypeptides are a family of cell wall-anchored adhesins that play a critical role in oral streptococcal adherence, colonisation and microbial community development. AgI/II polypeptide homologues have recently been discovered in pathogenic Streptococcus pyogenes (Group A Streptococcus [GAS]) and S. agalactiae (Group B Streptococcus [GBS]). GAS expresses one AgI/II protein, AspA, whilst GBS expresses four AgI/II proteins, BspA-D. Given the functional importance of this protein family in oral streptococci, we hypothesised that AgI/II adhesins may play a similarly critical role in GAS/GBS colonisation and pathogenesis. This project aimed to generate structural models for each protein and investigate the adhesive capabilities of AspA/Bsp proteins. SAXS and SANS data revealed an elongated fibrillar structure for AspA/Bsp proteins, similar to those structures already proposed for oral streptococcal AgI/II polypeptides SpaP and SspB. In addition, GBS proteins BspA and BspC were shown to promote binding to gp340 and saliva, comparable to AspA. These proteins also mediated interactions with C. albicans, a member of the resident microflora found in vivo. Such adhesive capabilities are likely to significantly influence colonisation by these pathogenic streptococci, and may represent novel targets for the control or prevention of GAS/GBS disease.

GM/17

Monitoring for re-evaluation of microbial criteria in foods for each food product

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Food standards established due to a variety of food accidents and food regulations constantly requested by consumer groups, microbial standards of the Food Code has been reviewed even from the time of notice, newly established or repeatedly revised then became the present status. Thus we need an overall review of the Food Code microbial standards. Even though the microbial contamination of a product is not even, to determine the entire safety and sanitation by one sample inspection, the test result is controversial, we need to review the introduction of a statistical concept to analyze several food samples (two class attributes plan, three class attribute plan), and the survey of feasibility and effectiveness for the change of theoretical specification (draft). Accordingly, this study investigated the indicator organisms (aerobic bacteria, coliforms, Escherichia coli) and food-borne illness organisms (Salmonellaspp., Staphylococcus aureus, Bacillus cereus etc.). This study reflected results for 100 samples gathered in South Korea. It was evaluated by the methods of 3M Petrifilm for aerobic bacteria, qualitative test for coliforms, E.coli and food-borne pathogen in food product. This results of this study will be used as the basic data to set the resonable microbiological criteria of Korea Food Code.

GM/18

DNA is an essential component of the *Campylobacter jejuni* biofilm extracellular matrix

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The majority of bacteria exist naturally in either single or multi-species biofilms. Biofilm growth leads to increased tolerance to starvation, antimicrobials and survival in food chain relevant conditions. *Campylobacter jejuni* is one of the leading causes of infectious intestinal disease in the developed world. We have previously shown that *C. jejuni* biofilm formation is increased in aerobic conditions, however relatively little is known about *C. jejuni* biofilm formation and its extracellular matrix (ECM) composition.

We have investigated the role of extracellular DNA (eDNA) as a component of the *C. jejuni* ECM. Using DAPI staining and GFP expressing *C. jejuni* NCTC 11168, we have shown that eDNA is a substantial component of the ECM. Degradation of eDNA in mature *C. jejuni* biofilms by DNase led to the removal of the biofilm with no loss of *C. jejuni* viability. Strain RM1221, which expresses extracellular DNases, did not form biofilm in static cultures, and co-incubation of RM1221 and pre-formed NCTC 11168 biofilms resulted in the degradation of the mature biofilm. Molecular investigation of this mechanism is currently under investigation.

Enzymatic treatment of biofilms in the food chain is becoming increasingly popular and this work highlights the potential effectiveness of these treatments against *C. jejuni* biofilms. Weakening of the ECM leads to dispersal of the biofilm, allowing more efficient sanitisation of food processing equipment and ultimately safer food for the consumer.

GM/19

Chicken juice is a conditioning matrix which allows more efficient attachment of *Campylobacter jejuni* to abiotic surfaces

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The majority of bacteria exist in either single or multi-species biofilms, leading to increased tolerance to starvation, antimicrobials and environmental extremes. One of the conundrums of *Campylobacter jejuni* as foodborne pathogen is its successful survival in the aerobic conditions in the food chain, despite sensitivity to atmospheric oxygen levels. We previously showed that *C. jejuni* biofilm formation is increased in aerobic conditions in standard growth media, here we build on this work by investigating biofilm formation in chicken juice, representing an *in vitro* model for food-chain relevant conditions on abiotic surfaces.

As crystal violet staining was not suitable for measuring biofilm formation in this model system due to high non-specific staining, we used a novel metabolic dye (TTC)-based staining method to measure biofilm formation. In an *in vitro* model system based on media supplementation and replacement with chicken juice, *C. jejuni* biofilm formation was increased significantly in aerobic conditions, when compared to Brucella medium. Using SEM imaging, we show that chicken juice is able to increase biofilm formation in *C. jejuni*, which is linked to the high protein content of the exudate, which may 'condition' the attachment surfaces.

As meat juices are found ubiquitously throughout the food chain, they may offer *C. jejuni*, along with other food-chain associated bacteria, a means of increased survival through enhanced biofilm formation. A greater understanding of bacterial survival mechanisms in food matrices will undoubtedly aid in lowering the burden of bacterial contamination of the food chain, and ultimately safer food.

SC
Systems and cells

SC/01

The ability of *Cronobacter sakazakii* clinical strains to translocate through Caco-2 and human brain endothelial cell lines

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Background Cronobacter is an opportunistic bacterial pathogen especially among infants and immunocompromised adults, and is associated with several infections including necrotising enterocolitis (NEC), septicaemia and neonatal meningitis.

Methods Translocation assay was applied to assess the ability of 13 C. sakazakii strains to translocate through two cell lines; Caco-2 and human brain microvascular endothelial cells (HBMEC). The trans-epithelial electrical resistance (TER) was measured to investigate the ability of these strains to disrupt the tight junctions.

Results 10/13 C. sakazakii strains translocated across Caco-2 and HBMEC cell lines. It was notable that the TER of Caco-2 cell line declined when infected by the invasive strains, indicating their ability to alter the tight junctions.

Conclusions Ten C. sakazakii clinical strains were able to invade and translocate through Caco-2 and HBMEC cell lines. Three strains did not translocate and were not from meningitis cases. The translocation ability of these strains indicating their ability to overcome the host barriers. Moreover, disrupting the tight junction will lead to cell line permeability allowing more bacterial cells to migrate causing more damage to the host.

SC/02

***Candida glabrata* HOG1 regulates proline uptake, but not glycerol synthesis, under osmotic stress conditions**

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The high osmolarity glycerol (HOG) response MAP kinase is highly conserved amongst many organisms, and has been described to regulate glycerol biosynthesis under osmotic stress conditions in the model yeast *Saccharomyces cerevisiae*. The deletion of *HOG1* from the closely related, yet pathogenic, *Candida glabrata* resulted in an expected reduction in osmotic stress tolerance. Surprisingly, the deletion mutant displayed glycerol induction and accumulation similar to the wild-type parental strain. We investigated whether an alternative compatible osmolyte was instead being regulated by *HOG1*. Wild-type *C.glabrata* was found to increase internal proline concentration under osmotic stress (0.5M NaCl), this increase was not observed in the Δ*hog1* mutant, or in proline drop-out synthetic media. This lead us to hypothesise that the proline increase was due to uptake rather than synthesis, and concordantly, the proline transport gene *PUT4* was found to be upregulated 8.6 ± 1.8 fold higher relative expression in wild-type compared to the Δ*hog1* mutant. While proline uptake is not widely described as an osmotic stress tolerance mechanism in yeast literature, there are numerous citations describing the importance of proline transporters for virulence in bacteria. We are currently investigating the potential overlap between stress induced proline uptake and virulence in *C.glabrata*.

SC/03

A functional genomics toolkit for the wheat pathogen *Mycosphaerella graminicola*

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A significant aspect of ensuring global food security will be to develop new control strategies for the most important cereal diseases. This project is designed to build a tool kit of functional genomics and cell biology procedures, strains and resources that will act as the platform for tackling one of the most serious diseases of wheat in Europe, Septoria leaf blotch, which is caused by the fungus *Mycosphaerella graminicola*. Specifically, we will deliver: (1) A fully sequenced *M. graminicola* ORFeome in a Gateway entry vector and a suite of optimised destination vectors; (2) a *M. graminicola* Δ*ku80* strain to facilitate large scale gene targeting projects; (3) a set of *M. graminicola* inducible promoters; (4) a suite of *M. graminicola* strains with fluorescently labelled compartments and cytoskeleton components; (5) techniques for analysing *M. graminicola* wheat infections at the cellular level. We are also carrying out a re-annotation of the *M. graminocola* genome sequence to aid our construction of the ORFeome library and transcriptional profiling to inform our analysis of the biology of plant infection. These tools will facilitate the rapid characterisation of the Septoria wheat blotch fungus and we will disseminate these resources to the plant pathology research community, the UK agricultural biotechnology industry and to international collaborators. In this way, we aim to develop a concerted research effort to understand this economically crucial pathogen and identify new targets for broad-spectrum cereal disease intervention.

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