MICROBIOLOGY SOCIETY

Annual Conference 2017

3–6 APRIL, EICC, EDINBURGH, UK

POSTER ABSTRACT BOOK

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Posters displayed Monday to Thursday

Virus Workshop: Antivirals and Vaccines
Virus Workshop: Clinical Virology Network
Virus Workshop: Evolution and Virus Populations
Virus Workshop: Gene expression and replication
Virus Workshop: Innate Immunity
Virus Workshop: Morphogenesis, egress and entry
Virus Workshop: Pathogenesis
Annual Meeting of Protistology-UK society: Intracellular infection and endosymbiosis within protists
Aquatic Microbiology
Cell biology of pathogen entry into host cells
Circadian Rhythms
Critical health challenges in medical mycology
Epigenetic and Non Coding RNAs in Eukaryotes
Synthetic and Systems Biology Approaches to Microbiology

Posters displayed on Monday and Tuesday

Geomicrobiology
Macromolecular Machines
Microbial Mechanisms of Plant pathology Prokaryotic Genetics and Genomics Forum
Prokaryotic Microbial Infection Forum

Posters displayed on Wednesday and Thursday

Anaerobes in infection
Environmental and Applied Microbiology Forum
Heterogeneity and Polymicrobial Interactions in Biofilms
Microbial Cell Surfaces
Microbial Genomics: Whole Population to Single Cell
Microbial Physiology, Metabolism and Molecular Mechanisms Forum

Virus Workshop: Antivirals and Vaccines

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Investigating membrane viral bending proteins in coronavirus replication.

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The coronavirus envelope spike (S) glycoprotein, a class I viral fusion protein, is responsible for the fusion between the membranes of the virus and the host cell. The fusion peptide has yet to be definitively identified, but bioinformatics analysis suggests that at least part of the fusion peptide is located near the amino terminus of the S2 region of S. Potential conserved membrane-modifying peptides were selected based on amino acid conservation, proximity to the membrane and/or Amphipaseek amphipathic helix prediction software. Peptides were tested for membrane-modifying activity in the presence of giant unilamellar vesicles consisting of DPPC, sphingomyelin and cholesterol. A conserved amphipathic helix motif near the amino terminus of the S2 region beginning with the residues **IEDLLF** showed the strongest membrane-modifying activity of the peptides tested, and were more active than an amphipathic peptide from *Influenza A virus* M2 protein that has been previously demonstrated to modify membranes. **IEDLLF** peptides from *Mouse hepatitis virus* and *Middle Eastern respiratory syndrome virus* proteins both changed the apparent size and shape of vesicle membranes in a manner consistent with membrane insertion. In contrast, regions bracketing the transmembrane region of S, the amphipathic fusion motor region and conserved motifs of S2 did not show membrane-modifying activity. Together these results suggest that the **IEDLLF** region that is conserved across the *Coronaviridae* inserts into host membranes and functions as the fusion peptide of the coronavirus S protein.

Virus Workshop: Antivirals and Vaccines

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Targetting the RNA Packaging Signal-Mediated Assembly of ssRNA Viruses with Drugs.

Amy Barker[1], Simon White[1], German Leonov[3], Shabih Shakeel[2], Sarah Butcher[2], Reidun Twarock[3], Peter Stockley[1]
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Secondary structures in genomic RNA (packaging signals (PSs)) provide scaffolding for cooperative coat protein (CP) recruitment and virus assembly (Stockley et al. 2013). Using bacteriophage MS2 as a model, RNA-binding ligands to its highest affinity PS (TR) were identified.

Mitoxantrone (MTX), a clinically approved anti-cancer agent, was found to bind tightly to TR, consistent with its known specificity for RNAs having a 5' adenosine bulge in the stem (Zheng et al. 2009). Both TR and genomic RNA-mediated MS2 reassembly are readily inhibited by MTX in vitro in a concentration dependent manner. MTX showed specific inhibition of phage infectivity in vivo, although repeated passages in the presence of drug resulted in recovery of wild-type titre. Sequence analysis of these "escape" mutants suggests that their principle target is the essential maturation protein-RNA contact, rather than PS-CP contacts.

We have begun to apply similar approaches to the human Parechovirus 1 system in which we have identified up to 60 copies of a PS sequence. The protein side chains that make up PS binding sites are absolutely conserved throughout the Parecho genus, suggesting that in this case the CP would be a good drug target. Molecular modelling of drugs from a clinically approved database identified a series of compounds that are likely to have affinity for the PS binding site. In vitro these ligands appear to block PS binding (Shakeel et al. 2016).These data suggest that it will be possible to develop specific drugs that target specifics of viral assembly involving RNA-CP contacts.

Virus Workshop: Antivirals and Vaccines

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A Preliminary Report: Cervical Human Papilloma Virus Genotype Distribution in Reproductive Aged Women Attending Primary Health Care in Urban Gambia

Haddy Bah Camara[1], Kumba Sun Mboob[2], Fatou Banja[2], Foday Ceesay[2], Mathew Anyanwu[3], Edward Wright[1], Patrick Kimmitt[1]
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Persistent infection with high risk human papilloma virus genotype plays a vital role in the development of most cervical cancers and Cervical Intraepithelial Neoplasia (CIN) among sexually active women worldwide. In the Gambia, cervical cancer is the most frequently diagnosed cancer amongst Gambian women representing about 30% of all registered female cancers. The quadrivalent HPV vaccine, which targets HPV genotypes 16, 18, 6 and 11 was introduced in urban Gambia in 2014. The aim of this study is to determine HPV genotype distribution and to determine the efficacy of the quadrivalent vaccine in this population. Sexually active reproductive women (N= 178; 20 - 49 years old) attending the polyclinic were enrolled. A designed questionnaire was administered; endocervical and high vaginal swabs were collected to determine HPV genotype distribution and co-infection with other genital STI pathogens. HPV was determined using the consensus primers PGMY09/11 targeting the late protein (L1) gene. HPV infection rate was 12.4% and the most prevalent high risk genotype was HPV 52. About 60% of participants infected with HPV were co-infected with *Ureaplasma*. Bivariate analysis shows that *Ureaplasma* infection, early sexual debut, Low level of education, and female circumcision were risk factors but not significantly associated with HPV ($p > 0.05$). This study is the first study carried out in urban Gambia and has demonstrated that the quadrivalent vaccine might not be adequate to protect this population from HPV infection as most high risk genotypes identified in this study are not targeted by the quadrivalent vaccine.

Virus Workshop: Antivirals and Vaccines

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Characterization of a novel hepatitis C virus vaccine candidate based on MVA expressing the nearly full-length HCV genome and lacking C6L vaccinia virus gene

María Quirós Marín, Juan García-Arriaza, Carmen Gómez, Carlos Óscar Sorzano, Mariano Esteban Rodríguez
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Background: Vaccines based in the Modified Vaccinia virus Ankara (MVA) expressing HCV antigens elicited T-cell immune responses in preclinical and clinical studies. However, MVA still contains several immunomodulatory genes that counteract the host antiviral immune response and deletion of some of them is a promising approach for the improvement of MVA-based vaccines.

Methods: To improve the immunogenicity of a previously described HCV vaccine candidate (MVA-HCV), based on MVA expressing the nearly full-length HCV genome, the C6L MVA gene, which encodes for an inhibitor of IFN-β was deleted. The resulting vaccine candidate (MVA-HCV ΔC6L) was tested in vitro and in vivo and compared to its parental virus.

Results: MVA-HCV ΔC6L expresses all HCV antigens and deletion of C6L had no effect on viral growth. Innate immune responses triggered by MVA-HCV ΔC6L in vitro showed a downregulation of cytokines and chemokines compared to MVA-HCV whereas a similar profile of recruited immune cells was induced in vivo. Furthermore, MVA-HCV and MVA-HCV ΔC6L induced similar levels of high, broad and polyfunctional HCV-specific CD4+ and CD8+ T-cell adaptive and memory immune responses in vaccinated mice. This response was mainly mediated by CD8+ T-cells, which were primarily against p7, NS2 and NS3 HCV proteins. Antibodies against E2 were also induced.

Conclusion: Deletion of C6L gene did not improve the immunogenicity elicited by MVA-HCV, probably due to a general blockade of immune response mediated by the HCV proteins. Thus, other strategies and prime-boost immunization combinations might be tested in order to improve the immunogenicity elicited by MVA-HCV.

Virus Workshop: Antivirals and Vaccines

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Antiviral Studies of Several Natural Extracts against Dengue Virus Serotype-2 Replication in vitro

Yasmina Zahra Syadza[1], Haryo Satrio Muhammad[1], Muhammad Arif Sanusi[1], Haryo Prakoso Adhi Purwanto[1], Catharina Nenobais[1], Kartika Laksmi Hapsari[1], Beti Ernawati Dewi[2]
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Background: This study was conducted by using *Callophylum canum, Swietenia mahagoni, Duria zybenthinus murr, Callophylum flavoramulum, Shorea sp.*, and *Kigelia Africana* to determine the effect of those extracts to DENV in vitro replication for antidengue candidate.

Method: The study was conducted by giving each natural extracts to both DENV serotype-2 infected/uninfected Huh7it-1 cell and DMSO as a control. Using MTT assay and focus assay, the CC_{50}, IC_{50}, and selectivity index (SI) of each extracts were resulted and converted as numerical data in SPSS and evaluated for its normality by Saphiro-Wilk test. For the results of statistically significant, one-way ANOVA or Kruskal-Wallis were used.

Results: The cytotoxicity effect of *C. flavoramulum* (473.5 µg/ml, $p<0.05$), *C. canum* (620.57 µg/ml, $p<0.05$), *K. africana* (CC_{50} = 439.12 µg/ml, $p< 0.05$), and *S. mahagoni* (434.46 µg/ml, $p<0.05$) were found low compared to control. The highest inhibitory concentration shown by *Shorea spp.* (IC_{50} = 23.33 µg/ml, $p <0.05$) and followed to lower concentration consecutively *K. Africana* (IC_{50} = 37.36 µg/ml, $p<0.05$), *C. flavoramulum* (IC_{50} = 41.74 µg/ml, $p<0.05$), *S. mahagoni* (IC_{50} = 68.97 µg/ml, $p<0.05$), *D. zybenthinus* (IC_{50} = 99.13 µg/ml, $p<0.05$), *C. canum* (IC_{50} = 123.96 µg/ml, $p<0.05$). *C. flavoramulum* and *K. africana* are the most selective extracts among others with SI value 11.33 and 11.75 respectively.

Conclusion: *C. flavoramulum* and *K. africana* extracts are potential to be used as antidengue to inhibit DENV serotype-2 than other extracts. Further study is needed to find the active ingredients and mechanism of inhibition stage of DENV.

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A novel approach in gene therapy by using directed evolution to generate antiviral drug

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Abstract

The coronavirus nucleoprotein protein (N) is an important component for both viral replication and transcription. Transient expression of N has been shown to increase the efficiency of infection and virus yield. We investigated the function of N by screening for dominant-negative N mutants, using a library of N variants constructed using error-prone PCR. The cytotoxicity of N variants was tested by MTT assay. Expressed N variants showed a range of effects ranging from the expected 10-fold increase in virus yield to 10-fold inhibition of virus growth. One particular N variant, mutant38, was non-toxic, but reproducibly inhibited virus growth. The potential to screen for dominant-negative N variants using cell survival was also assessed and mutant38 is found in live cells after several round. Together these results demonstrate that antiviral peptides can be generated by mutating an important viral accessory.

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A shift in HA/NA balance in pandemic H1N1 influenza virus

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Heamagluttin (HA) and Neuraminidase (NA) possess vital functions in the viral replication cycle to mediate entry and release from host cells. Live Attenuated Influenza Virus (LAIV) takes these two proteins from wild type seasonal influenza and so the growth characteristics of LAIVs will be directly impacted by the properties of the HA and NA.

The impact of the changes in HA and NA between pre-pandemic H1N1 and pandemic H1N1 (pdm09H1N1) on receptor binding and NA enzymatic activity was investigated.

HA binding properties of a selection of pre-pandemic and pdm09H1N1 LAIVs were measured by biolayer interferometry, using 6'sialyl lactosamine sugar. PdmH1N1 LAIV had qualitatively poorer binding, and decreased binding rates compared to pdm09H1N1 LAIV. NA activity of the same LAIVs was determined against MUNANA, a fluorescent small-molecule substrate, and also against fetuin, a complex glycoprotein, through the Enzyme Linked Lectin-binding Assay (ELLA). Pdm09H1N1 LAIV demonstrated markedly higher activity in both assays. By these measures the balance of binding of pdm09H1N1 HA and the activity of the NA on these LAIVs has shifted significantly from pre-pandemic levels and this has occurred alongside a decrease in observed LAIV and wild type potency.

However the measurement of NA activity has thus far been limited to non-biologically relevant substrates. We will present data using the lesser utilised Amplex Red kit, a multi enzyme assay that can quantify cleaved sialic moieties from varied biologically relevant substrates, including long chain sialic acids and mucous from primary human nasal epithelial cells.

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The use of primary human nasal epithelial cells in the selection of Live Attenuated Influenza Vaccine

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Lower than expected effectiveness with live attenuated influenza vaccine (LAIV) against H1N1 strains was observed in the US in recent seasons. This reduced effectiveness coincides with the introduction of the A/H1N1pdm09 virus strains into the vaccine. As part of the usual strain selection process, the replication kinetics of potential vaccine viruses are assessed in canine MDCK cells. However, based on these recent effectiveness findings, replication of LAIV has also been assessed in the human alveolar cell line A549 and in primary human nasal epithelial (HNE) cells cultured in transwells with a liquid air interface.

A549 cells and primary human nasal cells were infected with a diverse range of LAIV covering H1N1 (both pre and post pandemic), H3N2 and both B lineages. Following infection, virus was sampled from the supernatant (A549 cells) or apical surface (HNEs) of cells every 24 hours and the viral load quantified. The results show that the pdm09 H1N1 vaccine strains such as A/California/07/2009 and A/Bolivia/559/2013 had significantly reduced replication in human cells compared to pre pandemic viruses such as A/New Caledonia/20/1999 and H3N2 and B strains.

These studies suggest that reduced replicative fitness of A/H1N1pdm09 LAIV strains in human cells is a probable root cause for the observed reduced VE with LAIV in recent seasons. A possible explanation for this could be reduced human cell receptor binding; however, other mechanisms are also currently being explored. The data presented also highlights the value of the primary human cell model for future vaccine candidate selection.

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What are the Key Success Factors and Barriers for Translatability of Your Infectious Disease Research in Setting up Collaborations with the Industry/Corporate Businesses?

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Increasing cost pressure on healthcare systems, the requirement of publically funded academic research to demonstrate impact, depletion of well-validated drug targets for development of anti-virals/vaccines in the pharmaceutical sector and regular emergence of new infectious disease threats necessitates efforts for increasing strategic partnerships between industry/corporates and academic/higher education research institution's researchers.

What is the secret recipe for highly effective Academia-Corporate/University-Industry (A-C/U-I) research partnerships and what can researchers do to enhance the translational/commercial/societal benefits (impact) of their research were the questions that drove this research?

After in-depth interviews and rigorous qualitative thematic analysis of 14 stakeholders and Key Opinion Leaders from industry, academia and independent Organisations- this research demonstrates **high level scientific efforts, interpersonal skills, people competencies, organisational behaviour and leadership attributes as Key Success Factors** which are required to establish and manage A-C/U-I Strategic Research Partnerships (SRPs) effectively. SRPs require alignment of mutual complementary scientific interests and skills to co-design research with strategic partners- a concept that most organisations appear to be oblivious of. **Poor policies, lengthy administrative processes, lack of clear strategy on valuing research intellectual property and people without requisite attributes are the main barriers** that pose significant threat**.**

This research also reveals traditional approaches to measure success/outcomes pose significant risks which cannot be measured with traditional quantitative metrics. **Development of a single collaborative framework and mutual decision making process for high-order Strategic Research Partnership Management (SRPM) of A-C/U-I relations will be discussed- an area where Societies like Microbiology Society have a role to play in guiding members.**

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Receptor binding properties of pdm09H1N1 Live Attenuated Influenza Virus vaccine candidates

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Live-attenuated influenza viruses (LAIVs) need to be able to bind efficiently to both the α2,6-sialic acid (SA) receptors in the upper respiratory tract of patients and the α2,3-SA receptors found in embryonated chicken eggs for vaccine production. Egg-adaptation of the key 222/223 residues of the pdm09H1N1 HA from D/Q to G/Q or D/R has been previously shown to alter binding preference with the latter abolishing α2,6 binding.

Biolayer interferometry was utilised to determine whether pdm09H1N1 LAIV candidates carrying combinations of the 222/223 residues could bind to the α2,6-SA analogue 6'sialyllactosamine (6'SLN). All combinations of viruses from the 2014-2016 strain selection seasons were successfully rescued using reverse genetics. Rescued D/R viruses failed to bind to 6'SLN but G/Q viruses had improved binding to 6'SLN over the cell reference D/Q viruses. During the 2016-2017 strain selection season no pdm09H1N1 HA sequences were identified possessing the G/Q sequence. In addition, D/Q viruses routinely failed to rescue. The introduction of the G/Q sequence into these pdm09H1N1 viruses considerably improved rescue efficiency without hindering 6'SLN binding. In silico computational modelling of 2015 H1N1 viruses suggested that the improved binding of G/Q viruses to 6'SLN is due to a structural rearrangement of the binding pocket allowing the 223Q residue to form an extra hydrogen bond with 6'SLN.

The data presented here highlights the importance of understanding, at a molecular level, the receptor binding properties of cell and egg derived HA sequences, which will improve the rational design and selection of LAIV candidates.

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PSEUDOTYPING EFFICIENCY OF (RE-)EMERGING VIRUS ENVELOPE PROTEINS USING ALTERNATIVE CORES.

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The use of pseudotyping technology; the generation of replication defective versions of highly pathogenic viruses bearing native viral envelope proteins (VEP), can be employed to study early events in virus replication. Our group has spearheaded the use of pseudotyped viruses (PV) as surrogates to handling highly pathogenic viruses for serological studies. While our work has primarily focused on lyssaviruses and filoviruses, we have recently expanded this to include Nipah virus and Lassa virus.

To accommodate a broader range of user requirements we have incorporated new reporters and determined whether the disparate readout units of fluorescent and luminescent reporters, recorded as infectious units (IFU) and relative light units (RLU) respectively, can be correlated. Further, we have explored alternative methods of quantifying PV, measuring the number of particles, genome copies and reverse transcriptase (RT) activity, in addition to biological titre which is currently used. Our results showed measures of RT activity or genome copies with biological titres to be a good method for standardising assay input.

In addition, with a growing range of PV systems available it is important to determine which is the most appropriate for specific VEP. Using lentiviral (HIV), retroviral (MLV) and rhabdoviral (VSV) core pseudotyping systems we have shown that the efficiency of VEP incorporation into the PV envelope varies. Host innate restriction factors could also affect the choice of target cells. This work will help guide which combination of PV systems and cell lines are best to use for a range of VEP from (re-)emerging viruses.

Virus Workshop: Antivirals and Vaccines

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Predication of sofosbuvir response using a single nucleotide polymorphism of interferon lambda- 4 gene as a predictive factor

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Background: About 15% of the population in Egypt have chronic hepatitis C and over 90% out of them have HCV genotype 4. The aim of the present study was early predication of the efficacy of HCV treatment with sofosbuvir (sovaldi) and compare between the antiviral efficacy of dual (plus ribavirin (RBV)) and triple sovaldi combination therapy (plus pegylated interferon (PEG-IFN) and RBV). **Methods:** Blood samples of 100 patients chronically infected with HCV genotype 4 from different Egyptian healthy centers were used for DNA extraction and SNP genotyping. SNP genotyping for IFN-L4 at ss469415590 was performed by taqman probe assay. RT-PCR for HCV-RNA was carried out at the start and the end of treatment and twelve and twenty four weeks from the end of the treatment**. Results:** SNP genotyping showed that 13 patients carry ΔG/ ΔG , 28patients carry TT/TT and 59 patients carry heterozygote allele ΔG/TT. Clinical data showed that 8 patients stopped treatment, 70 patients were HCV negative after one month of treatment and all patients got negative at the end of treatment but 14 patients got relapsed. Only one patient developed hepatocellular carcinoma. **Conclusion:** IFNL4 ss469415590 variant is associated with various HCV clearance. This variant is associated with improved triple regimen therapy in Egyptian patients infected with HCV genotype 4.

Virus Workshop: Antivirals and Vaccines

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The Preclinical Characterisation of RV521, an Orally Bioavailable Inhibitor of RSV Fusion.

Stuart Cockerill[1], Rachel Harland[1], Sara Johnson[4], Neil Mathews[1], Michael Paradowski[2], Mark Peeples[4], Claire Scott[1], Geraldine Taylor[5], Michelle Thom[5], Elaine Thomas[1], Simon Ward[2], Daniel Watterson[3], Gareth Williams[2], Paul Young[3], Eddy Littler[1]
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Background: RSV therapies are being developed initially to treat infants and children where the duration and severity of the disease is most significant. In 'compromised' adult patients, effective remedies are of utmost importance. In the general adult population, treatment during a local epidemic would be used to prevent the spread of this life threatening infection. The longer duration of RSV disease in vulnerable populations supports a role for a small molecule in both treatment and prophylaxis regimens.

Methods : RV521 was identified at ReViral Ltd supported by a Wellcome Trust Seeding Drug Discovery award. Lead compounds were optimised utilising a cell fusion assay, virus infectivity assays and subsequent pharmacokinetic studies in vitro and in vivo with parallel toxicology studies and in vivo evaluations.

Results: RV521 was a potent inhibitor of the RSV fusion process (EC50 0.91nM) and both virus subtypes in plaque assay with an average EC50 of 3.47nM for subtype A (n=5) and 1.20nM for subtype B (n=4). In addition, RV521 was orally bioavailable in mouse (46%), rat (42%) and dog (44%). Efficacy was demonstrated in the primary well differentiated human airway epithelial and BALB/C mouse models of RSV infection where prophylactic administration at 50 mg/kg reduced lung virus titres by 98%. As a result RV521 was progressed into the pre-clinical evaluation phase.

Interpretation: RV521 is a potent fusion inhibitor of RSV subtypes A and B with a pharmacokinetic profile supportive of progression to clinical evaluation.

Virus Workshop: Antivirals and Vaccines

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Exploiting *Pichia Pastoris* for production of a virus-free vaccine against polio

Lee Sherry, Oluwapelumi Adeyemi, Danielle Pierce, Clare Nicol, David Rowlands, Nicola Stonehouse
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Poliovirus (PV) is highly transmissible and the known causative agent of poliomyelitis. Infection can result in an acute flaccid paralysis in up to 2% of cases and can be fatal. Integrated global immunisation programmes using live-attenuated oral (OPV) and/or inactivated PV vaccines (IPV) have systematically decreased spread and paved the way for eradication. Although PV is now only endemic in Afghanistan, Pakistan and Nigeria, immunisation will continue post-eradication to ensure against reintroduction of the disease. However, there are biosafety concerns for both OPV and IPV, both of which have the potential to reintroduce PV into the environment.

These concerns could be addressed by the production and use of virus-free virus-like particle (VLP) vaccines which mimic the ‘empty' capsids (ECs) normally produced in viral infection. ECs are antigenically indistinguishable from mature virus particles making them ideal vaccine candidates. However, they are less stable and readily convert to an alternative ‘non-native' conformation which can no longer induce protective immunity. Our group has recently published the production of thermally-stable mutant ECs of type-1 PV (PV-1) that are antigenically stable at temperatures above the conversion temperature of wild type virion. Here, we utilise these thermally stable mutants to produce VLPs from *Pichia Pastoris* through the heterologous expression of the PV-1 structural protein precursor, P1, and the viral protease, 3CD (which is necessary to ensure correct processing required for particle assembly). These results pave the way towards the production of safer vaccines for use in the post-eradication world.

Virus Workshop: Antivirals and Vaccines

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Unlocking the Potential of Host Cell Chloride Channels as Anti-Viral Targets during Human Respiratory Syncytial Virus infection.

Eleanor JAA Todd, Hessa Taqi, John N Barr, Jamel Mankouri
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Human respiratory syncytial virus (HRSV) is a non-segmented negative stranded RNA virus and is the most important viral agent of lower respiratory tract (LRT) infection worldwide. A recent meta-analysis reported that HRSV causes 34 million global LRT infections annually, with 10% requiring hospitalization, resulting in up to 199,000 deaths. In addition, recent evidence suggests HRSV infection early in life contributes towards increased incidence of adult asthma. There is no HRSV vaccine. The only viable treatment is prophylactic administration of an HRSV-specific antibody that is prohibitively expensive. A strong need for new anti-HRSV therapies remains.

Our recent work has shown that several segmented negative stranded RNA viruses require active cellular ion channels to complete their life cycles. HRSV, in common with many viruses, encodes a viroporin (the SH protein), implicating a requirement for electrochemical balance during virus infection. Whilst over 230 genes in the human genome encode ion channel subunits, no functional role of these key cellular proteins during the HRSV life cycle has been assigned. We therefore applied a comprehensive screening regimen to investigate a role for multiple cellular channel families during HRSV infection.

We report that HRSV growth is considerably reduced when chloride ion (Cl-) transport is blocked. Using an extensive panel of reagents to modulate the ≥40 genes that confer membrane Cl- conductance, we have identified the specific Cl- channels required for HRSV growth. These studies make a significant contribution to our understanding of HRSV-host interactions and have identified a potentially new druggable target to impede HRSV infection.

Virus Workshop: Antivirals and Vaccines

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A novel moo-noclonal antibody platform: from antibody to vaccine design, and back again.

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Background:
Most existing veterinary, and human, vaccines were developed empirically, but the field is moving towards rational vaccine design. Antibodies offer protection against viral infection, but not all antibodies are protective. Effective vaccine design requires a better understanding of the most protective determinants. Alcelaphine herpesvirus-1 (AlHV-1) causes malignant catarrhal fever disease in ruminants. The generation of a vaccine that protects against AlHV-1 would be commercially viable and highly desirable.
Methods:
Two 2-month old male calves were prime immunised with inactivated attenuated AlHV-1 in emulsigen adjuvant. After two weeks, a booster immunisation was given. Peripheral blood was then harvested a further two weeks later. Bovine peripheral blood mononuclear cells were then separated from whole blood by Ficoll gradient. Using a mixture of bovine specific, and species cross-reactive antibodies, combined with magnetic beads (Miltenyi) and FACS, bovine plasmablasts were single cell sorted into a proprietary cell lysis solution. cDNA was then produced, from which variable heavy and light domains were amplified, cloned into an IgG expression vector and expressed. Expressed monoclonal antibodies (mAbs) were tested for reactivity to AlHV-1 proteins, using a capture EIA, and for virus neutralisation, using a CPE assay.
Results:
Here we show successful isolation of vaccine induced AlHV-1 mAbs and describe their reactivity and neutralisation phenotypes.
Conclusion:
We have successfully established a novel and robust platform for isolating and interrogating mAbs from *Bos Taurus*. The platform will allow us to interrogate the antibody repertoire induced by a vaccine, which can be utilised to inform further rational vaccine design.

Virus Workshop: Clinical Virology Network

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Screening of Patients with Haematological Malignancies for Hepatitis B Core Antibodies: The Experience at University College London Hospital

Zaneeta Dhesi, Eleni Nastouli, Frank Mattes
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Background

Hepatitis B virus (HBV) reactivation in immunosuppressed patients is well recognised. We have audited against the NICE guidelines (2013) for Hepatitis B Core positive immunosuppressed patients. The aim of the audit was to identify whether HBV serological markers and viral load were performed and communicated.

Methods

All Hepatitis B core positive results between January 2015- December 2015 for haematology patients were extracted. Non-malignant patients were excluded. HBV serological markers and HBV viral load (HBVL) were recorded, alongside documentation of communication, and any prophylaxis used. Data were collected and analysed using Microsoft Excel.

Results

A total of 40 Hepatitis B core positive patients with diagnosed haematological malignancies were identified (52 samples). The mean age was 41 years (range 19-84). 18/40 (45%) were female and 22/40 (55%) male. 15/40 (37.5%) were patients undergoing hematopoietic stem cell transplantation (HSCT); and 26/40 (65%) were either on B cell depleting therapies and/or HSCT patients. All patients had an ALT result. All patients were HBsAg negative and none had a detectable HBVL. 31/40 (77.5%) had both anti-HBs and anti-HBcore results; 7/40 (17.5%) had e markers as well as anti-HBcore; 18/40 (45%) had both a HBVL and anti-HBcore. 5/40 (12.5%) had a complete set of results - anti-HBcore, anti-HBs , HBsAg, e markers and HBVL. Only 2/40 (5%) had documented evidence of result communication on our pathology system.

Conclusion

This demonstrates the need for comprehensive algorithms for HBV screening in this population at risk of reactivation. Downstream communication of results and follow up is key.

Virus Workshop: Clinical Virology Network

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Seroreactivity to three distinct regions within the hepatitis C virus Alternative Reading Frame Protein (ARFP / Core+1) in patients with hepatocellular carcinoma.

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Background: The alternative reading frame protein (ARFP/core+1) is encoded by a sequence overlapping the hepatitis C virus (HCV) core gene in the +1 reading frame. Its role in hepatitis C pathogenesis is unclear, although some observers relate its production to the development of hepatocellular carcinoma (HCC).

Aim: To determine whether ARFP is immunogenic in patients with HCV infection , identify at what stage of disease anti-ARFP first appears and whether changes in anti-ARFP titre correlate with disease progression.

Methods: Genomic RNA recovered from the serum of patients with genotype-3a HCV isolates were amplified by RT-PCR and sequenced using genotype-specific primers. Three peptides representing regions of ARFP, and one peptide representing an immunogenic region of the core protein, were designed based on sequence conservation and antigenic profile. Serum samples from HCV-infected patients at varying disease stages, including pre- and post-HCC diagnosis, plus healthy control sera, were tested for reactivity to these peptides using an antigen-capture ELISA.

Results: The ARFP amino acid sequences derived from HCV-infected individuals with HCC displayed a considerable degree of variability compared with a highly conserved core, as the ARFP phylogenetic analysis revealed greater inter-node genetic distances. Serum samples from HCV-infected patients with HCC reacted with all ARFP and core peptides, without a considerable difference in reactivity between peptides. No reactivity was detected in healthy controls.

Conclusion: Sera taken from patients with HCV-associated HCC possess antibodies exhibiting strong reactivity against ARFP and Core peptides using ELISAs. Data from patients at all stages of HCV infection will be presented.

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The Impact of Maedi-Visna on Breeding Flocks

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Maedi-Visna, also known as Ovine Progressive Pneumonia, is a chronic wasting disease of sheep and goats that is the result of infection with the small ruminant lentivirus (SRLV), maedi-visna virus (MVV). MV is 100% fatal upon appearance of clinical signs with no cure or treatment available. In consequence, farmers with high value livestock can sustain heavy losses. With regard to breeding rams, this can additionally result in the loss of valuable genetics. There is evidence that sexual transmission of SRLVs can occur in goats via intrauterine insemination, this route however bypasses several natural barriers of the reproductive tract and it is not known what the risk of this transmission route is via natural mating. The current study seeks to quantify the risk of MVV transmission from infected rams to ewes via intravaginal insemination as a proxy for natural mating. Semen was collected from 13 naturally MVV infected rams using an artificial vagina, pooled and used to inseminate 12 naïve ewes with a control group of 12 naïve ewes being mock inseminated. Following insemination blood was collected weekly from ewes for 7 weeks. From blood, RNA and DNA extracted for qPCR and ELISA. Ewes were sacrificed at 7 weeks post insemination with tissues collected for MVV testing. Preliminary results suggest a low risk of sexual transmission of MVV from known positive rams. Potentially providing a technique to salvage valuable genetics from infected rams whilst limiting risk of transmission.

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HCVGenoTyper: A computer program for accurate hepatitis C virus genotyping using high throughput sequences

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Accurate genotyping is essential for hepatitis C virus (HCV) treatment. It helps clinicians assess infection severity and guides them to administer suitable treatment. Here we present HCVGenoTyper, a computational tool to determine HCV genotyping using high-throughput sequence data. We have used well-characterised publicly available HCV sequences to develop a k-mer based classification method. Unique k-mers, 53bp long generated from the reference sequences were used as a training set. Test k-mers of the same length were generated from the high-throughput sequence data and rapidly screened against the training set using Judy dynamic arrays. The coverage and depth of each reference genome were calculated by counting identical k-mer matches. Following that, noise in the k-mer results was removed by eliminating low-coverage reference matches and genotypes were assigned to the test set based on statistical evaluation. We used publicly available 180 fastQ and fastA sequences including simulated mixed genotype sequences to evaluate the prediction accuracy of our tool. In all our tests the prediction accuracy of this method was very high (>99%). It correctly identified all single and mixed genotypes in the test sets as well as suggested an appropriate reference genome for the reference assembly. Moreover, the program was memory- and time-efficient. A run on a ~1M sequence dataset completed in ~ five minutes and consumed less than 1 Gb RAM on a BiolLnux server with 2Tb RAM 80 processing cores. Thus, we have developed a rapid genotyping method that accurately predicts HCV genotypes using high-throughput sequence data.

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HIV SCREENING: IT'S THE ELEPHANT IN THE ROOM

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According to Public Health England's 'HIV testing in England: 2016 report', 13,500 (95% credible interval 10,200-17,900) people were estimated to be unaware of their HIV infection. The UK National Guidelines for HIV testing, 2008 suggest that all adult patients with predefined clinical indicator diseases should be offered HIV testing.

We performed a series of clinical audits in 4 NHS Trusts across Northwest England to establish the HIV testing rates in patients with a number of clinical indicator diseases. In particular, we looked at patients with invasive pneumococcal disease, viral meningitis, cerebral abscesses, campylobacteriosis, salmonellosis and shigellosis.

The main finding of these audits is that the HIV status of a large percentage of patients with these indicator diseases is not known. Of the patients that had the test already or were offered the test, a high diagnosed prevalence of HIV was observed in some of these patient groups, especially in shigellosis.

Our audits demonstrate that testing for HIV in certain patient groups can lead to an increased number of early HIV diagnoses, therefore leading to better patient outcomes, lower treatment costs and reduced public health implications.

This audit specifically looked at the clinical indicator diseases with a bacterial or viral aetiology. It is our opinion that clinical microbiologists and virologists should incorporate this in routine clinical consultations and practice. All laboratory confirmed cases of these microbial indicator diseases should ideally include an automated laboratory comment suggesting HIV screening and should become part of management of these diseases.

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C-REACTIVE PROTEIN AS AN EARLY MARKER OF OPPORTUNISTIC INFENCTION IN HIV

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BACKGROUND-Opportunistic infections account for the majority of death in untreated patients withAIDS. CRP is a highly sensitive marker of infection & inflammation and its levels increase with infection.METHODS-The present Study was undertaken among 1oo HIV+ patients, at ART center Victoria Hospital Bangalore. With the informed consent of the patient, a generalized proforma was filled up consisting of patient's clinical presentation and diagnosis. Their CRP level and CD4 count were measured. RESULTS-56 HIV+ patients were asymptomatic and acted as control giving a negative test for CRP (<6mg/l), Showing no base line rise in CRP. Patients with infectious diagnosis showed a positive test for CRP, while patients on treatment were negative. CRP levels as high as 192mg/l were found in patients with endometrial cancer. Among the infectious cases, bacterial infection showed high level of CRP (mean 32mg/l) compared to viral/fungal infection (mean 9mg/l). Combinations of opportunistic infections produced a high level of CRP (mean 45mg/l). A graph of CRP along x-axis and CD4 count along Y-axis were plotted which showed a negative correlation ($r=-0.2324$, $p<0.01$ and $|z|=2.40$). From the graph, the CRP level at which ART can be started is >92.413mg/l [taking <200 (cells/? l)as the CD4 count at which ART is started]. Patients showing negative test for CRP need not be started with ART, as their CD4 count is found to be approximately 329 cells/?l.CONCLUSION-CRP level in HIV patients has a prognostic significance and can be used as an early marker of Opportunistic infections.

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Generation of Vesicular Stomatitis Virus (VSV) Pseudotypes for Serological Studies of Zika Virus

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Zika virus (ZIKV) is an emerging flavivirus transmitted by mosquitoes in the tropical and sub-tropical regions. Other notorious arboviruses from the same family include Japanese encephalitis virus, West Nile virus and Dengue virus. The co-circulation of ZIKV and other members of the flaviviruses in humans causes a substantial problem for accurate diagnosis due to extensive cross-reactivity between these viruses. Pseudotyped viruses (PVs) can detect virus-specific neutralising antibodies and do not require high-level biocontainment due to their single-round replication nature.

Producer (BHK21) cells are transfected to produce recombinant VSVs with plasmids encoding structural proteins of VSV: pBS-N-ΦT, pBS-P-ΦT, pBS-G-ΦT, pBS-L-ΦT and the VSV backbone pVSV-ΔG-luc in which the glycoprotein (G) gene was deleted and replaced with luciferase reporter gene. To generate pseudotyped VSV, this recombinant VSV is then used to infect target cells that are transiently expressing ZIKV glycoproteins. Transcription of the VSV proteins is under the control of T7 RNA polymerase promoter (T7 pol).

Different options for providing T7 pol were compared, including infection of the producer cells with fowlpox virus expressing T7 pol or use of a T7 pol-expressing plasmid. Performance of the VSV pseudotyped ZIKV in neutralisation assays will be validated by comparing with the results of neutralisation assays using live ZIKV.

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HEV GLUE: a new web-based tool for investigating the epidemiology HEV

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Introduction

In Europe, infection with hepatitis E virus (HEV) usually occurs in travellers returning from endemic areas. However, there has been a recent upsurge in HEV infections acquired via the consumption of undercooked food and also via transfusion of blood products. There are currently many deficiencies in our understanding of HEV epidemiology, including the distribution and diversity of circulating variants, and the dynamics of transmission within and between host species. We have therefore developed a web-accessible sequence database, HEV~GLUE, to support investigations of HEV epidemiology in Europe.

Method

HEV-GLUE was developed using GLUE, a software framework for capturing virus sequences along with their associated metadata. Hepatitis E sequences were downloaded from the NCBI Nucleotide database, including both whole genome sequences and sub-genomic regions. Metadata including country of origin, host species, collection year, sample source, and PubMed study ID were extracted from NCBI records and normalised. Metadata items were checked and corrected by hand, with missing items completed where possible.

Results

The web-based tool currently includes 13,281 sequences. Annotated sequences have been made publicly accessible via the HEV-GLUE web resource (http://hev.glue.cvr.ac.uk). This allows researchers to survey the diversity of published hepatitis E sequences based on their epidemiological characteristics.

Conclusions

Understanding the epidemiology of HEV and the genetic determinants of HEV infection severity will be greatly improved by basing phylogenetic studies on sequences linked with metadata, available from the HEV-GLUE database.

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The role of deep sequencing using amplicon approach in the virology diagnostic laboratories

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Background

Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV) circulate in the host as quasispecies. Patients may harbour minority variants that lead to drug resistance and may go undetected by population sequencing. In this study, we compared the detection of drug resistance-associated variants (RAVS) for HCV NS3 protease inhibitors, HIV-1 Protease inhibitors (PI), HIV-1 nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) by Sanger sequencing and Next generation sequencing (NGS) using Illumina technology

Method

A threshold of 20% was applied to compare the ability of both sequencing platforms to detect RAVs. The produced amplicons from 94 samples were processed using both Sanger sequencing and deep sequencing on the MiSeq platform. The target genes were HCV NS3 and HIV Pol genes.

Results

In general, NGS was able to detect more RAVs than the Sanger method. When applying the 20% threshold, good concordance was observed between both sequencing techniques in detecting RAVs. However, Sanger sequencing failed to detect RAVs with a frequency of less than 20% on most occasions compared to NGS which was superior in detecting minority RAVs.

Conclusion

In conclusion, NGS is a promising technique to be utilised for detecting minor resistance associated variants although the clinical relevance in diagnostic virology needs further investigation. Amplicon sequencing is readily automated and may offer an alternative to Sanger sequencing in reference laboratories.

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Development and Optimisation of Dried Blood Spot for the Detection, Genotyping and Epidemiological Study of HCV in Africa

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Hepatitis C is a global health problem affecting approximately 2.8% of the world population, almost 185 million people worldwide. Sub Saharan Africa has a prevalence of 5.3% with approximately 32 million people living with the virus. Despite a high burden of this infection in Africa, using classical serology for diagnosis and surveillance of infection has become a huge challenge. This is due to logistical problems such as specimen collection, transportation and storage conditions. The use of Dried Blood Spot (DBS) technology circumvents these challenges however, DBS technique has not been standardised. Here, we have developed a new DBS protocol for detecting and genotyping in molecular epidemiological studies of HCV. We also compared a two-step RT-PCR method with one step RT-PCR using 6 chronic HCV samples as a pilot study. Serum samples were amplifiable using 3 primer sets specific for NCR, HVR1 and NS5B regions of HCV. Conventional sera RNA extraction was used as standard in parallel with the DBS. Our results showed that the three regions (NCR, HVR1 and NS5B) amplified from DBS eluates at 20 µl RNA volume had the same band patterns on gel electrophoresis with the convectional sera samples. Furthermore, we observed that the two step RT-PCR method is much more sensitive than the one step RT-PCR assays. This study however validates the use of DBS with this protocol as an alternative method for conventional nucleic acid serology and it can be used for in-country molecular epidemiological analysis of African HCV infection.

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Laboratory Diagnosis of Viral Meningitis: Clinical Virology Network (CVN) Survey 2016.

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Background: A survey was developed to collate the approach and methods carried out across the UK CVN diagnostic laboratory service regarding viral meningitis diagnosis.

Methods: Information was collected on the type and diagnostic scope of PCR assays employed in 2016. Consistency in virus target and assay source between laboratories was sought, together with approaches to the clinical management of individuals with herpes simplex virus (HSV) meningitis. 30 completed surveys were received from national, regional and specialist virology centres.

Results: All laboratories routinely performed HSV type I, HSV type II, varicella zoster and enterovirus (EV) PCR. 48% of all diagnostic molecular assays performed on-site were developed in-house, 26% were commercially available PCR kits, and 26% were referred elsewhere. Multiplex PCR was utilised as the core method by 86%, however 10% of laboratories did not use an internal control.

The survey highlighted patient immunosuppression (100%) or travel history to a relevant country (73%), as primary considerations for an extended pathogen test request. 30% of respondents would recommend antiviral treatment if HSV DNA was detected, 46% would do so in some clinical situations, and the rest would not treat the patient.

EV (80%), followed by HSV type I (7%), or HSV type II infections (3%) were the most common causes of meningitis.

Conclusions: It was notable that so many laboratories used multiplex in-house assays, and that EV infections were the most common cause of viral meningitis. In addition, not all respondents agreed whether aciclovir had a place in the management of HSV meningitis.

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Dried blood spot testing for blood borne viruses in prison

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Blood borne virus (BBV) infections, are a sociomedical problem due to frequency of chronic infection, transmission and cost of treatment. BBV infections are significantly higher in the prison population, including difficult to bleed intravenous drug users (IVDU), and reduced screening compared to the community. The use of dried blood spots (DBSs) has the potential to hugely increase screening, diagnosis and treatment of inmates.

The project aims were to determine the efficacy of DBS testing in prisons to detect BBVs and generate a phylogenetic profile of the HCV populations to assess the extent of within prison transmission. Paired serum and dried blood spot samples were taken in participating prisons. HBsAg, HCV Ab, HIV Ab/Ag and Syphilis Ab serology was performed on an Abbott Architect and the sensitivity (currently 100%) and specificity determined. Sample and data collection is ongoing.

Sequence data from retrospective HCV positive samples that had been genotyped were used to assess current genotype prevalence in UK prisons. Small variations were found between prisons with genotypes 1a and 3a dominating.

This data will also be used to look at the phylogeny of HCV within prisons determine whether specific strains are circulating within the different prisons.

The results will be used to validate DBS testing on the Abbott Architect, encouraging DBS use and IVDU testing. This project will also assess if HCV infection is occurring within the prisons or represent community acquired infections, which may impact on prison behavioural infection control management.

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A study to determine the incidence of Mycoplasma genitalium, Mycoplasma hominis and Ureaplasma parvum/urealyticum in genito-urinary specimens, by RIDA®GENE panel Real-time PCR assay.

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Mycoplasma spp. and Ureaplasma spp. have been identified as normal flora in many sexually-active individuals. They have specifically been found in the genital tract of pregnant women, causing various symptoms including, dysuria, abdominal pain, itching, burning sensations and predominantly vaginal discharge.

Clinicians primarily test for Chlamydia trachomatis and Neisseria gonorrhoeae in the genito-urinary medicine (GUM) patient population. Mycoplasma/Ureaplasma are currently only tested for in symptomatic patients that test negative for chlamydia and gonorrhoea.

The aims of this project were to look at the incidence of Mycoplasma genitalium, Mycoplasma hominis and Ureaplasma parvum/urealyticum in the GUM patient population from a range of locations, using an Abbott m2000sp extraction and r-Biopharm RIDA®GENE STI panel real-time PCR assay.

207/423 samples tested positive, out of which 47% detected Ureaplasma spp, 11.8% M.hominis and 3.3% M.genitalium. The proportion of M.hominis positives match previous studies of around 10%. In this study 11.8% have M.hominis which can be a secondary agent in pelvic inflammatory disease and can cause infection in pregnancy. In 2015, the CDC labelled M.genitalium as an emerging issue, as it is seen in 30% of males with recurrent urethritis and in up to 30% of females with cervicitis. In this study, 21.5% of males were positive for Ureaplasmas spp. 88.36% of patients positive for Ureaplasma were asymptomatic, confirming that cases are undiagnosed.

The results obtained will contribute to recognising trends in high-risk groups and determine how many cases of Mycoplasma/Ureaplasma infections are being missed and inform future laboratory policy.

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Wrestling with the not so simplex

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Case report

Disseminated (mucocutaneous) herpes simplex virus (HSV) infection is rare in immunocompetent individuals. We describe an immunocompetent healthy man with perianal and digital HSV-1 who developed widespread mixed vesicular/maculopapular rash, hepatitis, meningism and lymphopenia. Sexual history revealed a long term homosexual partner.

HSV-1 was detected in blood and skin swabs and a diagnosis of disseminated HSV-1 infection made. Serial HIV tests were negative, and T cell immunodeficiency and immunoglobulins screen were normal. HSV-1 serology was positive 8 days after onset of symptoms raising the possibility of reactivation but not excluding primary HSV.

In light of his partner returning recently from Japan and this atypical presentation we considered the possibility the B_gK_L variant which has been associated with Kaposi varicelliform eruption (KVE) in sumo wrestlers' possibly through enhanced pathogenicity and an altered immune response.

The HSV strain was isolated in Vero cells and whole genome sequencing performed. *In silico* restriction digest analysis revealed results that were not consistent with the B_gK_L strain.

There was clinical improvement on IV acyclovir with resolution of rash, lymphocyte count and hepatitis one month later. However, there was some residual hearing loss in the left ear.

Conclusion

We did not identify any clear cause for this particularly severe HSV infection but it remains prudent to consider investigating for viral and host factors whenever an atypical presentation arises.

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Investigation of the role of gastroenteritis causing viruses in the asymptomatic neonatal digestive tract.

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Background

Viral gastroenteritis is a significant cause of morbidity and mortality across the world with an estimated 1.7 billion cases of diarrhoeal disease each year, many of these in the under fives. However little is known about the role of viruses in asymptomatic infants. This study endeavours to identify whether certain viruses associated with diarrhoeal disease are present in asymptomatic neonates

Methods

Faecal samples from 245 symptomatic 0-1 year olds, 80 symptomatic 0-5 year olds, and 113 asymptomatic neonates were collected from patients attending the Royal Infirmary of Edinburgh and analysed for the presence of Enterovirus, Parechovirus, Astrovirus, Sapovirus, Norovirus, Rotavirus, Adenovirus and Human Bocavirus by Real Time PCR..

Results

Enterovirus was the most commonly found virus in the symptomatic population, whereas parechovirus, astrovirus, and sapovirus were rarely found. Enterovirus was found in 18.6% of asymptomatic neonates. Furthermore, this study showed the presence of Adenovirus DNA in 27% of asymptomatic children, compared to 11% seen amongst symptomatic children. No Norovirus, Rotavirus or Human Bocavirus infections were detected in the asymptomatic cohort, but were detected in 2.8%, 4% and 4.62% of symptomatic patients respectively.

Conclusion

Adenovirus is the most prevalent virus found in the asymptomatic population. Enterovirus is the most prevalent virus found in both symptomatic and asymptomatic populations of children of less than five years of age. Due to the appearance of Enterovirus in both groups it cannot conclusively be linked to disease, and highlights that these viruses may form part of the natural microbiome in young children.

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Implementation of HIV-1 Next Generation Sequencing at Barts Health NHS Trust

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Background: The Wellcome Trust funded '**I**nfe**c**tion Resp**on**se through Virus Genom**ic**s' (ICONIC) study is a collaborative effort led by UCL involving multiple partners, including Barts Health NHS Trust. One component to the project aimed to investigate the utility of next generation sequencing (NGS) technology in the diagnostic virology laboratory.

Methods: NGS pipelines were developed for common human viral infections including HIV-1 at the Wellcome Trust Sanger Institute and tested using residual patient clinical extracts. These technologies were transferred into Barts Health NHS Trust including evaluating the role of NGS in HIV-1 genotypic antiretroviral resistance testing (GART).

Results: Specific modifications to the PCR and bioinformatics analysis were required in order to optimise the pipeline for HIV-1 diagnostics. Preliminary investigation has assessed the reproducibility of results and compared resistance mutation detection with the routine diagnostic Sanger sequencing.

Conclusion: Management of patients infected with HIV-1 requires GART. Classically, GARTs are performed using population based Sanger sequencing; however, this method will only detect resistance mutations that are present at 20% of the overall viral quasispecies. NGS provides a potential method for identifying minority variants below this threshold.

Initial findings show that the implementation of NGS in the diagnostic laboratory from the research environment is a realistic option, provided there is access to appropriate resources and support. Further streamlining and automation of the technology will be beneficial to this key development.

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Evaluation of an intervention designed to increase diagnosis and treatment of patients with hepatitis C virus infection within drug treatment settings

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Background

Identification and treatment of HCV-infected individuals is not straightforward in the high-risk group of people who inject drugs (PWID) attending drug treatment centres (DTCs). This study explored the effectiveness of a complex intervention within DTCs at increasing HCV diagnosis, referral, assessment, engagement and treatment of PWID via routine clinical pathways.

Method

We piloted a 12-month intervention within 3 UK DTCs which included appointment of a facilitator to coordinate a range of activities aimed at increasing diagnosis and enhancing patient referral. Five sites without any intervention acted as controls. The primary endpoint was clinical engagement with therapy, defined as patient had completed investigations including viral load and genotype, had udnergone assessment of liver disease stage and had received a consultation regarding their treatment options.

Results

Two sites have finished thus far. Data respectively show ~3 & 10 fold increases in referral rates, and 4 & 18 fold increases in attendance and levels of treatment engagement. Control sites changed little from baseline levels. The effective components of the facilitator-mediated intervention, and reasons for patient drop-out at all points along the care pathway will be presented.

Conclusion

The introduction of nurse facilitators within drug treatment settings increases testing and patient referrals and significant increases in engagement and treatment of PWID within routine clinical care pathways.

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Case Report: Native Kidney BK virus nephropathy

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BK virus causes an asymptomatic infection in childhood, leading to lifelong persistence in the kidney which can reactivate in immunosuppressed hosts. Bone marrow and renal transplant patients are at risk of disease, which may result in haemorrhagic cystitis, ureteric stenosis and nephropathy. We describe a case of BK nephropathy in a patient not considered to be in an 'at risk' group for BK disease.

This patient was diagnosed with Non-Hodgkin lymphoma in 2011, for which he received chemotherapy. He relapsed in 2014, and received his last round of chemotherapy in February 2015. He presented one year later with worsening renal function and tiredness. Although chemotherapy stopped 12 months prior to presentation, his counts (red blood cell, haemoglobin, haematocrit and lymphocyte) were intermittently low and he was receiving low dose IVIG. Obstruction was suspected and bilateral stents inserted, with no resolution. BK nephropathy was diagnosed when the cytopathic effect seen on routine histology prompted SV40 staining, supported by a high BK viral load in whole blood. This was an unexpected finding as more than 12 months had passed since his chemotherapy, and he had not received either a bone marrow or renal transplant. He was given Cidofovir and immunoglobulin. Despite these interventions, he went into end stage renal failure and commenced peritoneal dialysis. BK viral load eventually decreased to undetectable levels but with no return of renal function.

BK nephropathy should be considered in patients other than renal and bone marrow transplant recipients, even if not currently receiving immunosuppressive agents.

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Controls for the syndromic diagnosis of gastrointestinal infections

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Nucleic acid amplification is commonly used for rapid pathogen identification and has traditionally identified a single pathogen in a single sample. Recent development of syndromic panels, that can identify multiple pathogens in a single sample, has resulted in increased efficiency and also time and cost savings to the diagnostic laboratory. Considerable effort is taken to validate these assays yet effective standardisation is seldom undertaken that would assure the quality of data generated over time and between different laboratories.

Commercially available and in house qPCR assays vary both within and between laboratories in their content and sensitivity. Many laboratories introduce positive controls consisting of purified plasmid DNA. This represents an imperfect control because of differences in the quality of plasmid purification and its inability to control for the nucleic acid extraction step.

At NIBSC we have developed a multiplex run control that contains 18 gastrointestinal pathogens to facilitate standardisation of assays. The pathogens were selected following consultation with multiple laboratories. The pathogens are mixed, freeze dried and supplied to the end user whom can extract the control alongside clinical samples allowing for standardisation of the extraction procedure as well as the qPCR. The freeze drying excipient concentrations were optimised to maintain cake structure and pathogen stability.

The usage of this external run control will contribute to effective standardisation of diagnostic assays and reduce both intra- and inter laboratory variability of reported results and, as a result, support a more complete diagnostic picture for the clinician to interpret.

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HBV Serology Testing prior to B-cell Depleting Therapy

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Background

Best practice guidance issued by NICE recommends HBV serology testing prior to treatment with immunosuppressive therapy (IST) given the risk of viral reactivation. Patients found to have evidence of current or past HBV infection should be further assessed and considered for monitoring and prophylactic antivirals.

Methods

The records of all patients who were prescribed Rituximab or Ofatumumab (anti-CD20 antibodies) in the trust between November 2014 and October 2015 were retrieved. For standards to be met, results for both HBsAg and HBcAB needed to be available prior to start of IST. All patients with a positive HBsAg or HBcAb were reviewed if additional HBV DNA levels were measured. The HBcAb positive patients also required a documented anti-HBs result.

Results

497 patients were issued with anti-CD20 antibodies. 175 patients (35%) were appropriately tested. 2 patients tested HBsAg positive of which one had a quantitative HBV DNA test with both having onward hepatology follow-up. 13 patients tested HBcAb positive of which 9 had anti-HBs levels performed. 8/13 had a quantitative HBV DNA test performed. One patient who tested HBsAg negative but HBcAb equivocal was not followed-up and developed HBV reactivation.

Conclusion

Compliance with NICE guidance was poor. The absence of follow up of a patient with equivocal HBcAb has resulted in one serious adverse event. A specific trust policy modified from current NICE guidance has since been implemented, warning of the risks accompanied by a step by step algorithm of investigations and management to improve patient care.

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Evaluation of the One-step ELITech HEV ELITe MGB real time PCR on the ELITe InGenius ™ automated sample-to-result platform for qualitative detection of Hepatitis E virus RNA in serum.

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Hepatitis E is an emerging zoonosis in the UK. Hepatitis E cases now exceed Hepatitis A cases, and immunocompromised patients develop persistent infections requiring antiviral therapy. Molecular diagnosis is required to support serological diagnosis, and to detect and monitor infection in immunocompromised patients. We evaluated a novel commercial one-step HEV RNA assay, the HEV ELITe MGB real time PCR (ELITechGroup) in comparison with an in-house 2 step RT-PCR used in the PHE Virus Reference Department (VRD). Testing of a HEV genotype panel, a WHO International Standard, and two UK NEQAS samples indicated that the HEV ELITe MGB detected a broad range of HEV genotypes, with similar Ct values to the reference method. Parallel testing of serum from 31 patients originally investigated at VRD indicated 100% sensitivity and 90% specificity of the HEV ELITe MGB relative to the reference method. Two samples tested low positive by the HEV ELITE MBG only, both of which were HEV IgG and IgM positive and one of which originally tested positive at VRD. Thus the HEV ELITe MGB is of at least comparable sensitivity and specificity to the reference method. The HEV ELITe MGB has also a universal internal process control shared between all Pleiades MGB ELITech assays, allowing use of the same nucleic acid solution for DNA and RNA assays simultaneously. The test is performed on the ELITe InGenius, a clinical diagnostic sample-to-result platform which allows automated processing of 12 samples with 30 minutes hands-on time and total assay time of 3.5 hours.

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Study of BK Virus Epidemiology Using a Novel Genotypic Assay

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Background: BK Virus infection in childhood leads to latency in renal tissues which can be problematic in immunosuppressed populations. In renal transplant recipients, viral load monitoring and urine analysis for decoy cells predict development of BK nephropathy which is diagnosed on the basis of histopathological changes with coincident viral antigen detection in the tissue biopsy. The epidemiology of BK virus is studied by serological and genotypic methods. Seroprevalence studies show four serotypes with varying global distribution and genotypic assays based on the VP1 antigen follow serological groupings and permit subtype classification.

Methods: The Large T antigen (LTA) demonstrated potentially better subtype resolution than VP1 by *in silico* analysis of DNA sequences. Therefore a genotypic assay based on this region was developed and applied to patients exhibiting BK viremia at Barts Health NHS Trust. Statistical analysis was carried out to assess possible associations of genotype and development of BK nephropathy, alongside the study of known risk factors.

Results: The LTA assay exhibited robust subtype discrimination. Genotypes from within the renal transplant population reflected those commonly found in Europe with 1a, 1b1, 1b2 and IV being detected although the sample size precluded statistical association of nephropathy and specific genotypes. Known risk factors including the presence of decoy cells in urine and viral load at six months post-transplant were also significant predictors of nephropathy in this population.

Conclusion: The DNA sequence of the LTA region of BK virus can be used to determine genotype, facilitating studies on transmission and pathogenesis of the virus.

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Improved molecular detection and differentiation of avian paramyxovirus type 1 (APMV-1)

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Newcastle disease is a highly contagious avian disease caused by Newcastle disease virus (NDV), the virulent form of avian paramyxovirus type 1 (APMV-1), and is one of the most devastating diseases of poultry worldwide. A rapid, sensitive and specific means for the detection of NDV/APMV-1 is fundamental for the differentiation and control of this notifiable avian disease. Although several real-time RT-PCR assays exist for the detection of APMV-1, sensitivity and specificities are variable and benefit from regular optimisation. Here we describe a modification and improvement to previously published APMV-1 L gene RT-PCR and APMV-1 pathotyping RT-PCR assays (Fuller *et al.,* 2010; 2009). In order to assess assay performance following modification, a panel of 60 APMV-1 and 18 non-APMV-1 strains comprising both virulent and avirulent strains were selected to represent the full range and genetic diversity of APMV-1's, along with those that are currently circulating in Europe. Both assays demonstrated an increase in diagnostic sensitivity and specificity, and offer a rapid and sensitive means for the detection and pathotyping of avian paramyxoviruses.

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Reference materials for viral metagenomics

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Viral metagenomics has great potential in the detection of novel or highly divergent viruses that may be missed by sensitive but highly specific PCR-based assays. The power of the method has been demonstrated in studies that detected porcine circovirus contamination in a human vaccine and numerous case reports where clinical metagenomics has contributed to diagnosis of infections. Evaluation and optimisation of methods and definition of limits of detection for viral metagenomics assays is complicated by the lack of a pre-defined target. Reference materials containing defined quantities of representative virus classes will permit development and comparison of methods, and allow for limits of detection to be defined for particular virus types. We recently evaluated a candidate reference material in a multi-centre collaborative study and found dramatic differences in the design of laboratory and informatics methods, their ability to detect viruses, and wide variation in the proportion of sequencing reads assigned to each virus. We are currently designing a second generation reference material specifically designed for viral metagenomics. It is anticipated that the material will be directly applicable to adventitious virus detection in biological medicines, and may also prove useful in clinical metagenomics.

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Using a high dynamic range 4th generation HIV diagnostic assay to identify recent HIV infections - a retrospective study of 209 samples collected from individuals with newly identified HIV infections over 26 months.

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Background

Determining HIV incidence is essential for surveillance, intervention and prevention strategies. The HIV-1 LAg-Avidity EIA (Sedia ™) is an HIV incidence assay based on the functional antibody avidity or binding strength. Our aim was to investigate whether the relative light unit (RLU) values of a commercially available routine diagnostic test, the Abbott ARCHITECT HIV Ag/Ab Combo assay, could identify an infection as recently acquired or as a long standing infection.

Methods

All first time samples from individuals who tested as HIV-1 positive were referred for HIV-1 avidity over a 26 month period (July 2013- September 2016) at a South-East London hospital. Data collected included date of sample, testing history, ARCHITECT HIV Ag/Ab Combo, immunoblot, LAg-Avidity, subtype results and any available clinical information. A recent infection was defined as an LAg-Avidity result =/< 1.5 and a long-standing infection as >1.5.

Results

39 (19%) of 209 individuals had a recent infection as per the LAg-Avidity assay and the median ARCHITECT result was 62 RLU (mean 147, range 6-869) compared to a median of 631 RLU (mean 632, range 27-1130) for samples with a long-standing infection. Further tests are being carried out on 15 (7%) samples which could have been misclassified.

A majority of samples were subtype B (39, 45% n=88). The incident group included subtype B, C, CRF01_AE and CRF02_AG demonstrating that non-B subtype recent infections were identified.

Conclusion

The results indicated that the ARCHITECT could be used to stage HIV recent infection, which is important for surveillance and prevention strategies.

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Establishing a WHO International Standard for Zika virus antibody

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NIBSC is developing an International Standard for Zika virus (ZIKV) antibody to monitor the performance of diagnostic assays and aid vaccine research, as part of the World Health Organization's (WHO) response to the 2015 ZIKV outbreak in Southern America and to provision for any further outbreaks.
Although infection symptoms are usually mild, the risk of Guillain-Barré and other neurological disorders in adults, and microcephally in neonates has led to an increased need for correct diagnosis of zika virus infection. While the preferred Zika diagnostic assays used during the outbreak have been PCR-based methods, blood serological diagnosis is more appropriate if testing is over seven days from onset of symptoms. However it can be difficult to distinguish between ZIKV and other flavivirus infections such as dengue virus (DENV) due to cross-reactivity of antibodies. A reference standard would assist in the development of improved assays.
Candidate materials were donated from commercial organisations and health institutes; these underwent preliminary testing by in house anti-ZIKV antibody ELISA and ZIKV PRNT and by commercial DENV ELISA. Based on a consensus obtained from the results, materials were put forward into a panel. A collaborative study has been organised with laboratories worldwide.
Based on data gained from the collaborative study, one antibody sample will be recommended for endorsement as International Standard for Zika virus antibody by submission of a report to the WHO Expert Committee on Biological Standardization in October 2017.

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Adoption and validation of real-time RT-PCR protocols for sub-typing European swine influenza viruses in the United Kingdom

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Swine influenza is a viral infection contributing to respiratory disease and economic loss in pigs. Three sub-types ($H1_{av}N1$, H1N1pdm09, $H1_{hu}N2$) of swine influenza A viruses (swIAVs) including reassortants are currently found in UK pigs. Diagnostic tests capable of rapidly identifying swIAVs sub-type are important and assist surveillance, epidemiological investigations and vaccination decisions. Traditional sub-typing relies on virus isolation (VI) followed by serological HA and NA sub-typing which is laborious and depends on live virus being successfully isolated. Real-time RT-PCR (rRT-PCR) has recently improved the sensitivity and speed of swIAV sub-typing. Simplex rtRT-PCR assays for detection of H1 ($H1_{av}$ and $H1_{hu}$), or H3, and duplex assays for detection of N1 ($N1_{av}$) or N2 were successfully evaluated on egg-amplified viruses to achieve sensitive and specific identification of HA and NA genes of swIAVs from enzootic European lineages. Their performance was then assessed directly on clinical field material from which no live swIAV had been isolated. H1N2 and $H1_{av}N1$ swIAVs have been identified in VI-negative, untyped influenza A-rRT-PCR (M gene) positive swabs and tissues (n=68, 40% full HA/NA, 34% partial HA or NA, 26% no result) submitted to the UK swIAV surveillance project, 2012-2016. During this time, 41 H1N1pdm09 swIAVs were also specifically detected by rRT-PCR. These tests can more rapidly sub-type previously uncharacterized swIAV strains from field material without VI or nucleotide sequencing. This is particularly useful where material contains non-viable or limited live virus. However, maintaining VI capability and a swIAV isolate archive are essential for ongoing surveillance and research.

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Evaluation of lyophilised NAT run control materials for quality control of molecular syndromic diagnostic panels

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Background

Diagnosis of communicable diseases in clinical settings is rapidly evolving towards a 'syndromic' approach. This involves simultaneous molecular testing for a large number of organisms, creating the need for multiplex NAT run controls to assure the quality of assays. In order to provide suitable CE-IVD marked multiplex working reference materials, two multiple run control reagents have been developed to assure multiplex assays that diagnose respiratory disease and meningitis.

Materials/methods

Comprehensive panels of bacterial and viral agents that cause respiratory disease and meningitis were selected and created. Conditions for optimal freeze drying were investigated including the use of trehalose, mannitol, sorbitol, albumin and glycerine excipients. Stability of lyophilised material stored at -70°C, 4°C and ambient temperature were compared and data analysed by ANOVA.

Results

Assessment of pathogen stability identified 2% trehalose for respiratory virus and 2% sorbitol for respiratory bacteria and all meningitis agents as the optimal lyophilisation matrix. Products were stable at ambient temperature for logistical purposes as well as 7 days post-reconstitution at 4°C. Presence of whole viral and bacterial agents resembling a clinical sample allows controls to be employed as extraction and amplification quality control reagents aiding compliance with the requirements of ISO 15189.

Conclusion

Multiplex NAT reagents represent a cost-effective option for inclusion as a run control for multiplex assays to diagnose respiratory and meningitis disease. These new panels complement other multiplex working reagents previously produced at NIBSC.

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Albumin-bilirubin score within decompensated patients receiving direct acting antiviral therapy within the Expanded Access Programme

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The albumin-bilirubin (ALBI) score is a validated measure of liver function. We aimed to determine the course of liver disease (ALBI score) during and after antiviral treatment in patients with life-threatening HCV-related liver disease.

We included 626 patients treated for advanced liver disease in the NHS England Expanded Access Programme. We determined the mean change in ALBI score between start of treatment and the last measurement (mean time 38 weeks). Modelling was performed using multivariable logistic and Cox regression with forward stepwise selection of variables.

The treated population had an improved ALBI score on average, with a mean improvement of -0.27. Patients achieving an SVR 12 weeks following treatment had a significantly higher improvement (p=0.0031) 0.31) than those who did not (-0.14).

For ALBI measured at baseline, survival and time to clinically significant deterioration was significantly higher with better ALBI grades. For each unit increase in ALBI at baseline there was a near twofold risk of death hazard ratio (HR)=1.91 (95% CI 1.29-2.83).

Predicting death using multivariable logistic regression led to a model containing ALBI, genotype and gender, with a discrimination (area under ROC curve) of 0.73. For death within the first six months the variables were ALBI and genotype with an AUROC of 0.67.

Treatment of decompensated patients with DAAs improved ALBI score more in patients achieving an SVR than those who did not. ALBI score is a moderately good predictor of survival at baseline and this may be improved by a monitoring of ALBI over time.

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Using metaviromics as a novel tool to detect pathogenic viruses in the environment

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Environmental virus populations are vastly understudied in comparison with bacterial populations, due primarily to the absence of a universal marker gene. Screening for potential pathogenic viruses in environmental reservoirs and distribution systems such as wastewater effluents into rivers, coastal areas, and shellfish beds is currently performed with (RT-)qPCR for specific viral groups. While this technique can be very sensitive for known viruses, it cannot detect novel viruses or diverging genogroups. This study aims to develop and evaluate metaviromics as a new screening approach for both established and potentially novel pathogenic viruses in the environment.

Wastewater, surface water and sediment samples were taken in the Conwy river system in Wales. These were concentrated using tangential flow filtration and PEG precipitation, followed by isolation of the viral fraction by filtering and nuclease treatments. The viral RNA was extracted using the PowerViral Environmental RNA/DNA Extraction Kit (Mobio). Sequencing libraries were constructed with the NEBNext Ultra Directional RNA Library Prep Kit and sequenced on a HiSeq 4000 at the Centre of Genomics Research (University of Liverpool). This yielded between 3 and 110 million reads per sample.

The majority of sequencing reads were unknown/unassigned, as is universally found in environmental metaviromes. The known RNA viral fraction contained signatures belonging to the families *Virgaviridae*, *Nodaviridae, Reoviridae* and *Picobirnaviridae*, all of which contain pathogenic viruses for either plants, humans or animals. The abundances of the viral groups changed across samples, which warrants further spatial and temporal investigation.

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The spread of DWV and other RNA viruses between honey bees and their wider insect communities

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Throughout the world biodiversity is under threat and over the last few decades many pollinators including the honey bee have been experiencing significant losses. A major factor in their decline has been the spread of positive strand RNA viruses. Most notably for honey bees has been Deformed wing virus (DWV) which although widely studied in association with its vector the parasitic Varroa mite, still causes a major problem for beekeepers and further work is required if we are to discover a way to protect the bees. Although DWV is now known to be a generalist insect virus the extent of any potential spread beyond honey bees is as yet unknown. Furthermore although the majority of RNA viruses most commonly infecting honey bees have been known for some time, there are many more viruses including some we are only just discovering now, for example our recently discovered Moku virus, lurking within insect communities with the potential to spell further disaster for honey bees and their wider insect communities.

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Investigation of hantavirus prevalence in wild rodent population in the United Kingdom.

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Hantaviruses are a diverse group of RNA viruses, belonging to the Bunyaviridae family. They are primarily rodent-borne and transmitted into humans through the inhalation of aerosolised urine and faecal matter of infected animals. In their reservoir species, infection results in a persistent, yet asymptomatic infection. In humans, hantavirus infection can lead to hantavirus haemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS).
There have been multiple serologically confirmed cases of hantavirus infection within the UK, though the exact causative species is often not confirmed. In 2011 & 2012, 2 independent HFRS cases led to the isolation of distinct Seoul virus strains in wild and pet rats. Serological screening of human cohorts has indicated a significant exposure to hantaviruses within the UK. However, the identity and prevalence of these viruses within rodent populations is currently poorly understood, which this study aims to redress.
Samples were collected from rodents caught in Leicestershire, England. The initial screening process used 2 Step RT-PCR to screen organs from 72 rats, 224 mice and 12 field voles. Degenerate pan-hantavirus primers were used to amplify sections of the RNA polymerase gene. All rat and mice samples were negative; whilst hantavirus was detected in a single field vole. Sequencing of a 452bp fragment identified the isolate as Tatenale-like Hantavirus.
This represents the second identification of the Tatenale-like virus in the UK, extending detection beyond north-west England. The genome is currently incomplete and further work is required to characterise the virus and any potential zoonotic capability.

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Searching for dsRNA viruses

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Double-stranded RNA viruses are gaining more and more attention. For example, viruses of the family Totiviridae are found in ever greater numbers not only in fungi, but also angiosperm plants, in marine and terrestrial arthropds, and in single-cell human parasites such as Leihmania, Giardia,and Trichomonas. In some fungi, the viruses reduces the virulence of the fungus, in some species of leishmnai and in Trichomonas vaginalis, the virulence or resulting clinical pathology of the host is markedly increasd. Here we report a quantitative comparison of three methods to detect dsRNA viruses. RT-PCR based on degenrate primers for RNA-dependent RNA polymerase is compared with an ELISA based on dsRNA-detecting antobodies and with next generation sequencing of total RNA. The sensitivity of PCR methods is traded for its specificity, the cost of next generation sequencing is traded for price, while the ease of ELISAs for detection limits.

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Studies on Beauveria bassiana small narna-like virus, the smallest known virus

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Fungal viruses have been described in a wide range of fungi and are classified into eleven major families. The family *Narnaviridae* constitutes the simplest, non-segmented, unencapsidated positive-stranded RNA viruses that range from 2.3 to 3.6 kb in size and are further classified into two genera: genus *Mitovirus*, which are fairly common, and genus *Narnavirus*, which consists of only 5 members. We recently discovered a novel narna-like virus in the entomopathogenic ascomycete *Beauveria bassiana*, named Beauveria bassiana narna-like virus (BbSNLV). Cloning and sequencing revealed that BbSNLV is 1,689 bp in size and encodes a 58 kDa polypeptide, which contains motifs characteristic of RNA dependent RNA polymerases (RdRPs) including the conserved GDD motif. Phylogenetic analysis indicated that this polypeptide is distantly related to the RdRPs of the narnaviruses. BbSNLV was also shown to support to the replication of two non-coding satellite or defective interfering RNAs, derived from and reducing accumulation of the parent viral RNA. These subviral RNAs are common in the *Narnaviridae* but restricted to the genus *Mitovirus*. The BbSNLV RNA was over expressed using an in vitro transcription assay; in parallel the RdRP was over expressed in Escherichia coli and purified as a His-tagged protein using Fast Protein Liquid Chromatography. RNA-protein electrophoretic mobility shift assays confirmed the interaction between the BbSNLV genome and the RdRP, while the structure of the RdRP is currently being determined using X-ray crystallography. To our knowledge, BbSNLV is unique, the smallest known virus and the first narnavirus isolated from a hyphomycete.

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A structural and functional analysis of the non-primate hepacivirus (NPHV) 5' untranslated region

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NPHV is the most closely related virus to HCV, and is proposed to have diverged from HCV within the last 1000 years. Studying viruses which share a close evolutionary relationship, yet differ greatly in their ability to cause disease, provides a unique opportunity to facilitate comparative analysis of both replication strategies and pathogenic mechanisms. We previously demonstrated that, like HCV, the NPHV 5'UTR functioned as an IRES (Stewart et al, 2013), here we set out to undertake a more detailed structural and functional analysis of the 5'UTR.

To map the secondary structure we utilised 2'hydroxyl acylation and analysis by primer extension (SHAPE). This showed a similar structure to HCV but with an additional long 5' stem loop (SLI). The SHAPE data was used to guide a mutational analysis. The translational phenotype of these mutants was analysed and revealed a complex set of structural requirements for NPHV IRES function; for example mutation of a conserved GGG motif within SLIII (G310A/G312U), which has been shown to ablate HCV translation, severely impaired IRES activity. However, a deletion of the first two stem loops (SLI+II) was able to rescue translation of G310A/G312U. We also demonstrated that the liver specific miR122 positively upregulates translation from the NPHV IRES.

In conclusion, we have determined the structure of the NPHV 5'UTR and demonstrated that SLIII is crucial for IRES function. We propose that the NPHV IRES interacts with host ribosomes in a similar fashion to HCV, however SLI may play a novel negative regulatory role.

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Genetic and antigenic diversity of H9N2 avian influenza viruses prevalent in poultry in Pakistan

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H9N2 avian influenza viruses are enzootic among domestic poultry throughout Asia and northern Africa where they impose serious economic burden and have the potential to contribute to the generation of novel zoonotic viruses. Vaccination programmes to reduce their impact in poultry have been implemented in many countries, including Pakistan. However, efficacy of vaccines has been repeatedly compromised due to rapid virus evolution and emergence of antigenic variants. To address this we undertook targeted surveillance of H9N2 viruses infecting poultry in the Punjab province of Pakistan. Characterisation of these viruses indicates that the haemagglutinin gene is G1 lineage while the internal genes have differentiated to yield a variety of unique sub-genotypes. Antigenic cross-reactivity analysis using a panel of G1 lineage antisera showed some isolates to have marked antigenic diversity with up to 8 fold reduction in haemagglutination inhibition compared with Pakistani isolates from 2008, indicating that vaccines derived from older viruses may not cross-protect against recent variants. Furthermore, the potential of these viruses to cause zoonotic infection, by assessment of receptor-binding properties, showed that a single isolate had much greater binding potential for human-like receptor analogues. This work concludes that currently prevalent H9N2 viruses infecting poultry in Pakistan have undergone considerable genetic and antigenic drift. Consequently, the current vaccine formulation prepared using older viruses may not induce adequate protective immunity in poultry flocks and the observed emergence of an isolate with preference for human-like receptors raises the possibility of increased numbers of zoonotic infections.

Annual Conference 2017
EICC, Edinburgh
3-6 April

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Mycobacterium tuberculosis WhiB1 forms a nitric oxide-sensitive complex with the major sigma factor, σ^A

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Production of a Specific indirect ELISA for the detection of IgG to Zika virus

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Zika virus (ZIKV) diagnosis is primarily performed by rt-PCR; however this is hampered by the relatively short viremia present in clinical samples. Therefore serological ZIKV diagnosis would be preferential, but with this comes the issue of serological cross-reactivity with other endemic arboviruses especially Dengue virus (DENV). Currently only one authorised serological assay for ZIKV diagnosis is available and this has known issues with cross reactivity.
At the National Institute for Biological Standards and Control (NIBSC), we have been endorsed by the WHO to produce serological standards for ZIKV. To facilitate the characterisation of patient antibodies; we have produced an ELISA for the detection of IgG to ZIKV in serum or plasma. This was developed using the materials available to us at NIBSC as well as produced monoclonal antibodies. Cross reactivity to other arboviruses, specifically all 4 serotypes of DENV, and Japanese encephalitis virus (JEV) was tested using the International Standards available at NIBSC, with no significant cross-reactivity having been identified. The samples we have available for standards production will be used to compare the ZIKV IgG ELISA, plaque reduction neutralisation test (PRNT) assays and other commercial ELISAs.
The development of this ELISA shows that it is possible to produce specific diagnostic reagents for ZIKV IgG. This assay could potentially be applied as a diagnostic tool to determine exposure to ZIKV. The assay has also been used to characterise the convalescent samples that will make up a panel for use in a collaborative study to produce the first international standard for ZIKV.

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Next generation sequencing assessment of genetic stability of rotavirus vaccine virus in vaccinees in the UK.

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Rotavirus is the main cause of severe diarrhoea in under-5s worldwide. Vaccination with a two-dose oral live attenuated monovalent vaccine was introduced into the UK childhood immunisation programme in 2013, significantly reducing rotavirus disease in the population. As a live attenuated RNA virus vaccine, there is a potential for accumulation of mutations during the manufacturing process or shedding in vaccinees. To assess vaccine virus genetic stability, we first evaluated single nucleotide polymorphism (SNP) loci using next generation sequencing in two stages of the manufacturing process. Further, we sequenced RNA obtained from vaccinee stool collected post-vaccination at various time points including those of anticipated peak shedding. Data were aligned to reference standards and mutations called if there were >100 mean reads present at a mean frequency ≥1% in at least 2/3 replicates. In ten vaccine samples, we identified 13 SNP loci at consistent frequencies across independent samples, suggesting that the vaccine is stable. Preliminary data from early time-point samples from two vaccinees suggest the presence of a SNP locus against the Genbank reference standard (JX943613) in VP6 also found in vaccine stages at a frequency of 10%, indicating that the virus shed at early time points is likely the vaccine virus. So far, there are no additional SNPs although ongoing studies may yield further variant loci. Those present at a frequency ≥50% will be considered worthy of further study since they may represent a reversion to wildtype virus, which could pose a health concern in the population.

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Bat-plex: A multiplex neutralisation assay for bat-borne viruses

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A common thread uniting several major viral outbreaks in recent years has been the potential link of each to a bat reservoir. Studies monitoring viral infections in wild bat populations have identified serologic evidence for infection of bats with a variety of viruses with zoonotic potential. Despite the continual emergence of novel pathogens, large-scale systematic monitoring of bats has not been carried out. The potential for so many viral spillover events originating from bats, and due to the difficulty in obtaining serum from such small and highly mobile host animals means that protocols that minimise the amount of serum used in serological studies are a high priority for future research. The use of pseudotyped viruses (PV) to study seroprevalence in bats offers significant potential improvements in the efficiency of serological assays.
The development of panels of PV based on bat-borne viruses has presented the opportunity to develop efficient multiplex assays to detect neutralising antibodies that may indicate historical infection with a virus. This system makes use of different reporter genes expressed by PV bearing the glycoproteins of different viruses. It is, therefore, possible to use a single serum sample to perform a serological assay with multiple different viruses. In this study, we demonstrate the use of an Ebola PV-expressing emGFP, a Rabies PV-expressing tRFP and a MERS-CoV PV-expressing firefly luciferase, incorporated into a multiplex assay allowing serological screening for all three viruses to be conducted on a single serum sample.

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Rapid generation of polymorphic herpes simplex virus populations in cell culture

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Large dsDNA viruses are characterized by a low level of variability during viral replication due to the use of viral or host high fidelity DNA polymerases. Many viral genes are directly involved in viral pathogenesis and modulation of the host immune response; being essential a high degree of fidelity during viral replication. Herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2) are prevalent human pathogens of clinical relevance that establish latency in the nervous system and encode immune modulatory activities to evade the immune response.

Isolation of five plaque-purified HSV-1 and HSV-2 clones and subsequent sequencing of their viral genomes by next generation sequencing (NGS) showed genetic variability at the level of single nucleotide polymorphisms (SNPs). Many of these SNPs were already present in the parental viral stocks but others were introduced de novo, in coding and non-coding regions, showing either low frequencies in the population (not fixed SNPs) or high frequencies, close to the 100% (fixed SNPs). These results suggest the generation of HSV-1 and HSV-2 viral diversity during virus replication in cell culture.

The virulence of two viral clones of each HSV type with the lowest non-synonymous SNP number were tested in mouse models (BALB/cByJ), showing differences in survival and signs of infection compared to the corresponding parental stocks. To confirm whether genetic variability is generated in cell culture, we have performed additional passages of these viral clones in cell culture and sequenced their genomes by NGS.

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Reassortment Phylodynamics of H5 Highly Pathogenic Avian Influenza

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In 2014/2015 a highly pathogenic avian influenza (HPAI) H5N8 strain reached Northern Europe and North America from Asia via migrating wild anseriform birds, and again in Autumn/winter 2016 there have been outbreaks of H5N8 in Europe, with wild bird carriage implicated. In both cases, the hemagglutinin (HA) was from H5 clade 2.3.4.4, which has circulated with several neuraminidase partners in the last 10 years including N2, N5, N6 and N8 (H5Nx). This is in stark contrast to the limited reassortment in other clades of highly pathogenic H5N1. Here the effects of transmission through anseriform or galliform populations, neuraminidase reassortment, and HA evolution are investigated using sequence data and phylodynamic modelling.

H5, N6 and N8 sequence data were obtained from the NCBI Influenza Virus Resource. Time resolved Bayesian phylogenies were created, and discrete trait models for subtype, host-type, location and multibasic cleavage site motif were applied in order to infer ancestral states, and phylogenetically corrected correlation between traits. This showed acquisition of new neuraminidase types occurred readily from co-circulating low pathogenic strains, and that the reassortments occurred predominantly in anseriformes. Sites under diversifying selection were detected but were not strongly correlated with subtype or host changes. Overall phylodynamic analyses shows HPAI H5Nx is spreading and evolving in wild anseriformes, and is able to easily spill over into domestic flocks where the populations interact.

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Using next generation sequencing to investigate the mechanism of infectious bronchitis virus attenuation

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Vaccines against the coronavirus infectious bronchitis virus (IBV), the causative agent of the economically important avian infectious bronchitis, are produced by serial passage of field strain virus in embryonated eggs. This passaging introduces genomic mutations in the virus ultimately leading to viral attenuation. The mutations that create viral attenuation however, have not been established. M41-CK, a pathogenic strain of IBV, was passaged in four independent replicates over one hundred times with the aim of generating a virus attenuated in chickens. Using 454 pyrosequencing, the viral genomes of the starting inoculum and the final viral isolates were sequenced and compared with clear diversity being observed that would otherwise be undetected using consensus sequencing. From an originally heterozygous population, the majority of minor variants were lost in the final inoculum with the exception of three variants occurring at a frequency of 0.3243, 0.8072 and 0.2360 in nsp4, spike and E protein, respectively. Mutations at these three sites increased to an average frequency of 0.8406, 0.5036 and 1.000, respectively, for isolates in which the mutation was present. All four viral isolates contain a greater number of variable nucleotide positions with the nucleocapsid and 3' UTR having the highest rate of substitution per nucleotide. For all polymorphic sites identified, the majority (65%) were nonsynonymous. This information is being used to inform future work using an established reverse genetics system with the ultimate aim of developing rationally attenuated viruses.

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Molecular pathogenesis of H7N7 LP-HPAIV transitioning during UK outbreaks: 2008 and 2015

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Avian influenza (AI) is a notifiable avian disease (NAD) and a severe threat to the poultry industry. The United Kingdom (UK) has had periodic outbreaks of low pathogenicity (LP) and highly pathogenic (HP) AI. During 1959-2016, 12 HPAIV and nine LPAIV (subtype H5 and H7) outbreaks occurred. Notably, these included two LPAIV to HPAIV H7N7 mutational events during 2008 and 2015 in layer chickens. Three other UK chicken H7 LPAIVs did not mutate (2006-N3/2007-N2/2015-N7). The two LPAIV to HPAIV mutation events generated A/chicken/England/11406/2008 (Banbury, 2008) and A/chicken/England/26352/2015 (Preston, 2015). Evidence for initial H7N7 LPAIV entry followed by mutation to HPAIV was derived from disease patterns (egg-drop and mortality), pathogenesis, seroconversion along with the timing and proportions of H7N7 HPAIV shedding at the epidemiological units within the chicken premises. Furthermore, haemagglutinin (HA) sequencing of specimens from Banbury (2008) revealed three HP polybasic cleavage site (CS) variants: PEIPKRKKRGLF / PEIPKKKKRGLF / PEIPKKKKKKRGLF, and one LP-CS: PEIPKKRGLF. Preston (2015) had two HP-CS: PEIPRHRKGRGLF / PEIPRHRKRRGLF, and one LP-CS: PEIPKGRGLF. We have employed a Banbury 2008 reverse genetics system in an *in ovo* model to investigate the genetic and phenotypic LPAIV to HPAIV change. Further studies are underway in attempts to understand the drivers (viral/host) of evolution for viruses that mutate from LPAIV to HPAIVs to improve detection and control of NAD.

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Identifying unusual enteric viral infections from hospital patients with diseases of unknown origin in Vietnam

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Vietnam is a potential 'hotspot' for emerging zoonotic diseases, due to prevalence of practices allowing close contact between humans, livestock and wildlife. The Vietnam Initiative on Zoonotic Infections (VIZIONS) is a multidisciplinary surveillance project which aims to identify diseases of unknown origin (DUOs) in hospital patients and individuals at high-risk of zoonotic pathogen transfer. Between 2012 and 2016, 3200 patients with acute diarrhoea admitted to hospitals in five regions of Vietnam were recruited to the study, upon which they completed a questionnaire and had a stool sample collected. Samples from a subset of patients (n=270) were analysed using RT-PCR and a Luminex xTAG Gastrointestinal Pathogen Panel (GPP). A clinical DUO was defined as a patient in which no known pathogen was found using either method, of which 58 patients were identified. We used a metagenomics approach to identify enteric viruses present in 58 patients with clinical DUOs according to RT-PCR and Luminex. We also assessed the potential for identifying patients with DUOs using statistical analysis of demographic, clinical and behavioural data from questionnaire responses. In this way, we selected 30 patients with clinical symptoms and behaviour which may be associated with unusual enteric viruses. Samples from these patients also underwent metagenomic sequencing. This was successful at capturing pathogens missed by both PCR and Luminex and is a useful method for identifying previously unknown viruses.

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The ICTV Online (10th) Report: making virus taxonomy more accessible

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Virus classification is important because it provides a framework for understanding the astonishing diversity of viruses. The authoritative source of classification information is the Master Species List maintained by the International Committee on Taxonomy of Viruses (ICTV). This list is supplemented by the ICTV Report, the individual chapters of which are written by groups of experts in each virus family. The 1st Report appeared in 1971 as a slim volume, whereas the 9th Report, published in 2012, reached a total of 1327 pages. As the Report has become larger and more expensive, it has become increasingly inaccessible to the virological and educational communities, particularly in developing countries. A grant from the Wellcome Trust is now supporting online, open access, chapter-by-chapter publication of the Report on the ICTV website. In addition, useful chapter summaries are being published in the Journal of General Virology as ICTV Virus Taxonomy Profiles, which also provide the means of citing the full chapters. The move to a web-based format will allow the inclusion of in-text citations, sequence alignments and other supplementary materials, and will make it easy for chapters to be updated on a rolling basis. Through this new publication format, the ICTV will be able to respond more quickly to discoveries in virology, and the resources it produces will become fully and freely accessible to the scientific community and the public at large.

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Epstein-Barr virus – via EBNA3A and EBNA3C – represses the plasma cell differentiation pathway during the establishment of latency in B cells

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Mature human B cells infected by Epstein-Barr virus (EBV) become activated, grow and proliferate. *Ex vivo* infection results in continuously proliferating lymphoblastoid cell lines (LCL) that carry EBV DNA as episomes, express nine latency-associated EBV proteins and phenotypically resemble antigen-activated B-blasts. *In vivo* such B-blasts can differentiate into memory B cells, where lifelong EBV persistence is established. Three related viral latency-associated proteins EBNA3A, EBNA3B and EBNA3C are transcription factors that regulate several cellular genes. EBNA3B is unnecessary to establish LCLs, but EBNA3A and EBNA3C are required to sustain proliferation by repressing the expression of tumour suppressor genes. We have found, using EBV-recombinants where both EBNA3A and EBNA3C can be conditionally inactivated that – after an initial proliferation phase – infected primary B cells express elevated levels of plasma cell (PC) differentiation associated factors, including the cyclin-dependent kinase inhibitor (CDKI) $p18^{INK4c}$ and the transcription factor BLIMP-1. About twenty days after infection without functional EBNA3A and EBNA3C, around 20% of cells have a PC-like phenotype. ChIP-seq and ChIP-qPCR indicate that in LCLs, repression of *CDKN2C* ($p18^{INK4c}$) and *PRDM1* (BLIMP-1) transcription results from direct binding of EBNA3A and EBNA3C to regulatory elements at these gene loci and produces stable reprogramming. Furthermore, it is not possible to de-repress $p18^{INK4c}$ or BLIMP-1 – in newly infected cells or conditional LCLs – when the activating ligand is removed. Together these data suggest that EBNA3A and EBNA3C have evolved to prevent default PC differentiation after activation by EBV, thus favoring long-term EBV persistence in memory B cells.

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Evidence for Origin and Interspecies Recombination of Canine Respiratory Coronavirus based on Phylogenetic Analyses

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Although canine respiratory coronavirus (CRCoV) is an important respiratory pathogen and has been prevalent in many countries, to date only one complete genome sequence of CRCoV (South Korea strain K37) has been obtained and genome-wide analysis was rarely conducted. The small sample and limited genomic characterization have prevented further analyses so far. Herein, we reported a unique CRCoV strain, denoted strain BJ232 derived from a CRCoV-positive dog with mild respiratory infection. Phylogenetic and recombinant analyses showed that CRCoV-BJ232 strain belongs to the most recent common ancestor branch of CRCoV, and the previously reported CRCoV-K37 strain results from interspecies recombination between bovine coronavirus (BCoV) and CRCoV-BJ232. CRCoV-K37 strain occupied an intermediate phylogenetic position between CRCoV and BCoV. In detail, in the S gene recombinant strain clustered with CRCoV-BJ232, and in orf1ab, HE, M and N genes recombinant was closely related with BCoV. The interspecies recombinant events between CRCoV-BJ232 and BCoV may have implications for the transmissibility of CRCoV, and all of these findings provide further information for origin study of CRCoV.

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Investigating the function of adenoviral early region oncoproteins

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Adenovirus (Ad) early region genes can cooperatively transform human, and rodent, primary cells. The ability of Ad E1A, E1B-55K, E4orf3 and E4orf6 oncoproteins to promote cellular transformation resides in their capacity to target cellular proteins such as CBP/p300, pRB and p53. It is also apparent that Ad oncoproteins employ numerous strategies to selectively activate and/or evade host cell genotoxic stress response pathways during infection. Thus, Ad has evolved to bypass, or inactivate, host cell cycle checkpoints and DNA damage response pathways that would otherwise initiate cell cycle arrest or apoptotic programmes in the infected cell. In this regard E1B-55K, E4orf3 and E4orf6 function either individually, or in combination, and engage with the ubiquitin-proteasome pathway to target proteins such as p53, MRE11, TOPBP1, TIF1□□ Daxx, TFII-I and DNA ligase IV for 26S proteasome-dependent degradation. Recent studies from our laboratory have been investigating further the function of the E1B-55K, E4orf3 and E4orf6 oncoproteins. Results from these studies will be presented here.

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Evolutionary distinct influenza A viruses elicit different cellular responses in equine cells.

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Influenza A viruses (IAVs) are significant pathogens of humans and animals whose main natural host is considered to be wild waterfowl. Infection and transmission in new hosts (i.e. viral emergence) requires highly specific interactions between virus and host proteins. However, how an avian virus adapts to a mammalian host is not yet clear.

We hypothesized that mammalian adaptation of an avian-origin IAV would involve changes in virus-host interactions that would result in more efficient viral replication as well as more effective means to counteract the cellular response to infection.

To test this hypothesis, we studied the transcriptome of equine cells infected with evolutionary distinct H3N8 influenza A viruses (H3N8 IAVs). H3N8 IAVs are avian-origin viruses that have circulated in horses for over 50 years, providing us a natural model system to study the successful interspecies transmission and post-transfer adaptation of an avian-origin IAV to a mammalian host.

Equine infected cells were RNA-extracted at different times post-infection for RNA sequencing and transcriptomics analysis. Sequencing data was analyzed using CuffDiff program and differentially expressed (DE) genes identified. IPA software was used to determine the canonical pathways in which DE genes were involved.

Our results showed that infection with evolutionary distinct H3N8 IAVs affects different intracellular pathways, allowing us to identify potential mechanisms that led to the adaptation of AIV to the horse. In turn, our findings could provide insight on general strategies employed by avian influenza viruses to establish in mammals.

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The Role of Translation Termination Factors in Foot-and-Mouth Disease Virus 2A Peptide Driven Translational Recoding.

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2A/2A-like peptides are short sequences (20-30 amino acids) encoded predominantly within open reading frames (ORF) of RNA viruses including foot-and-mouth disease virus (FMDV). 2A peptides drive a non-canonical translation, in which the nascent chain is released from the ribosome at a sense (proline) codon, followed by continued translation to generate a separate downstream protein, initiated from the same proline codon. This may indicate that translation termination factors (eRFI/3) are involved in this translational recoding event, despite the absence of a stop codon. The aim of this study is to investigate the role of eRFs in the 2A reaction in *Saccharomyces cerevisiae* strains, expressing a range of mutant eRFI/3. Results obtained confirmed that reduced activity of eRF1/3 leads to defects in the 2A reaction. The inhibition of the 2A activity did not strongly correlate with the effect that mutations have on termination at stop codons. In particular, several mutations within the NIKS motif, which is essential for stop codon recognition, had minimal effect on the 2A reaction, this is consistent with mRNA recognition not playing a key role in the reaction. In further experiments, Rli1 (ABCE1) and eIF3j, further important factors in termination/ribosome recycling, were also found to influence the 2A reaction. In summary, these observations provide evidences supporting recruitment of eRFs to the ribosome to drive the non-canonical termination event that releases the first part of the 2A reaction.

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Accurate characterization of the chicken IFITM locus using MiSeq and PacBio sequencing technologies

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Interferon inducible transmembrane (IFITM) proteins are effectors of the immune system widely characterized for their role in restricting infection by diverse enveloped and non-enveloped viruses. The chicken IFITM (chIFITM) genes are clustered on chromosome 5 and to date four genes have been annotated, namely chIFITM1, chIFITM3, chIFITM5 and chIFITM10. However, due to a low number of sequencing studies focusing on this locus, accurate characterization has so far proven problematic. To address the paucity of information on the chIFITM genetic locus we have sequenced a bacterial artificial chromosome (BAC) containing the chIFITM locus, defined as inclusive of the two flanking genes ATHL1 and B4GALNT4. To generate an accurate consensus sequence, we utilized the sequencing data obtained using both Illumina MiSeq and PacBio RSII sequencers. Here we describe the quality of the genome locus assembly in terms of coverage distribution and mapping accuracy against either chromosome 5 of Gallus gallus reference genome or the consensus sequence obtained from de novo assembly of PacBio sequencing reads. The latter was also used as a scaffold for the re-alignment of RNA-seq data downloaded from the European Nucleotide Archive (ENA) to investigate immune-relevant cell type and tissue type expression patterns of different chicken breeds and other commonly used avian cell lines. Together this provides a definitive description of the important of the genomic locus at the nucleotide level which is necessary for future comparative genetic and transcriptomic studies.

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Remodelling events at the nuclear envelope during KSHV lytic replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a gammaherpesvirus responsible for the most common tumour in HIV-1 infected individuals in Africa, Kaposi's sarcoma (KS) 1. KSHV is a DNA virus that exhibits a biphasic lifecycle with a dormant latent period and a productive lytic cycle. KSHV replicates and packages its genome within the nucleus of host cells and therefore must traffic its RNA and proteins through the nuclear envelope. The nuclear envelope is a complex structure and interface composed of nuclear pore complexes, the nuclear lamina, and the nuclear membrane.
Increasingly, it has been shown that a range of viruses specifically target components of the nuclear envelope to aid with viral replication and progeny release. Notably, herpesviruses have been shown to remodel the nuclear pore complex to enhance viral replication2. Herein we have utilised a quantitative proteomic approach to analyse changes in nuclear envelope components during the KSHV lytic replication cycle. We identify significant changes to levels of the nucleoporin, Nup98 and subsequent validation highlighted the importance of Nup98 during viral infection. In addition, we observe significant changes to nuclear envelope-associated ESCRT-III proteins and continuing studies are investigating the significance of virally induced changes at the nuclear envelope and their wider significance on viral replication and virion release.

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Gene expression profiling of different avian cell types infected with IBDV vaccine strain

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Infectious Bursal Disease Virus (IBDV) is a birnavirus of economic importance to the poultry industry that has a tropism for B cells, which reside in the bursa of Fabricius (BF). Previous studies have investigated host gene expression during IBDV infection using an immortalised fibroblast cell-line (DF-1), *ex vivo* dendritic cells, and whole BF tissue. However, no study has characterised host gene expression in B cells. In this study, we compared the replication kinetics of IBDV strain D78 in four cell types: the DF-1 cell-line, primary chicken embryonic fibroblast (CEF) cells, an avian leukosis virus-induced immortalised B cell-line (DT40), and primary B cells that were extracted from the BF, sorted, and cultured *ex vivo* in media supplemented with CD40 ligand. RNA was extracted from each culture at 6, 18 and 24 hours post infection and the viral genome copy number determined. Samples were normalised to a house-keeping gene (TBP) and expressed relative to mock controls. All four cell types supported the growth of D78 with fold changes in viral genome copy number of 10^6, 10^5, 10^1 and 10^2 in DF-1, CEF, DT40 and *ex vivo* B cells respectively, at 24 hours post infection. In a separate experiment, the expression of cellular genes in infected *ex vivo* B cells was analysed by microarray and several interferon-stimulated genes, including IFIT5, MX1 and STAT1, were significantly up-regulated compared to mock-infected cells. Data will be presented comparing the host cell gene expression between the four cell types during IBDV infection.

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The Impact of *African swine fever virus* on host microRNAs

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African swine fever virus (ASFV) is the etiological agent of African swine fever (ASF), a highly contagious haemorrhagic disease of swine. Since 2007 the virus has spread throughout the Caucasus and Eastern Europe, entering the European Union in 2014. With mortality rates as high as 100% in domestic herds and no vaccine or treatment available, further outbreaks could have devastating effects on the European pig industry. ASFV is the only member of the Asfarviridae, though shares a number of biological characteristics with the Poxviridae. Both virus families have large, double-stranded DNA genomes that encode a number of homologous proteins including those essential for viral replication and transcription within the host cell cytoplasm. For example, sequence alignment has revealed that the ASFV encoded protein C475L is a homolog of Vaccinia virus (VACV) VP55, the catalytic subunit of the viral poly(A) polymerase. Infection with VACV disrupts the host microRNA (miRNA) system by a mechanism of polyadenylation and degradation, mediated by VP55. The aim of this study was to investigate whether ASFV C475L shares the ability with VACV VP55 to manipulate host miRNAs. Multiple methods were used to overexpress C475L in a variety of mammalian cell lines. However, there was no evidence that ASFV C475L has the ability to polyadenylate miRNAs or induce their degradation.

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The Chikungunya virus non-structural protein 3 (nsP3) alpha unique domain has multiple functions during the virus lifecycle.

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Chikungunya virus (CHIKV) is a re-emerging arbovirus causing fever, joint pain, skin rash, myalgia, and occasionally death. Antiviral therapies or safe, effective vaccines are urgently required. This project seeks to investigate the function of the CHIKV non-structural protein 3 (nsP3) in virus replication to identify targets for antiviral intervention and means of rational attenuation for vaccine development.

Firstly, a series of mutations in the central region of nsP3, also termed the alpha unique domain (AUD), were generated in the context of a CHIKV subgenomic replicon. Analysis of the phenotypes of these mutants revealed that AUD plays an essential role in CHIKV RNA replication. Intriguingly, some mutations exhibited species specific phenotypes - replicating in either mosquito or mammalian cells. Others showed different phenotypes that were consistent with a role for the AUD in counteracting host antiviral responses such as RNAi or cytoplasmic innate immune sensing.

The panel of AUD mutations were also introduced into an infectious CHIKV construct and the phenotype analysed in mammalian cells. These results confirmed the replicon data, and intriguingly revealed that one of the mutants (E225A) could replicate but had a defect in the production of infectious virus in Huh7 cells.

There has been no reports on AUD function so far. Our data reveal for the first time that the AUD may be a pleiotropic protein domain, functioning in both genome replication and virus assembly, as well as playing a role in counteracting host defences in both mammalian and mosquito cells.

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Investigation into the roles of human cytomegalovirus long non-coding RNAs

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Long non-coding (lnc) RNAs are defined as transcripts of over 200 nucleotides that do not encode proteins. With the advent of high-throughput sequencing, it has become apparent that lncRNAs are prevalent throughout animal and plant transcriptomes, where they are emerging as providing a crucial layer of gene regulation in a wide range of important cellular processes, including proliferation, differentiation, migration and apoptosis. Similarly, human cytomegalovirus (HCMV) encodes four major lncRNAs (RNA2.7, RNA1.2, RNA4.9 and RNA5.0) that are likely to have important functions: they are largely or entirely free from overlapping protein-coding regions, their sequences are among the most highly conserved parts of the HCMV genome, they constitute the great majority of polyadenylated viral transcripts in HCMV-infected cells, and they have evolutionary counterparts in other betaherpesviruses. Although some of the functions of these lncRNAs have been elucidated or suggested, much remains unknown. We are investigating the functions of RNA2.7 and RNA1.2 initially through the construction of deletion mutants in HCMV strain Merlin. By examining the transcriptomes and proteomes of the mutants, we have identified a number of differentially regulated cellular and viral genes. We are currently validating these target genes and investigating how their regulation may contribute to HCMV infection.

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Determining a high-resolution structure of FMDV 3D^{pol} fibrils by cryo-electron microscopy

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Foot-and-mouth disease virus (FMDV) is the causative agent of one of the most economically-damaging infections of cloven-hooved animals. FMDV is a positive-sense, single-stranded RNA virus in the family Picornaviridae and, although vaccines are available to prevent outbreaks, there are a number of caveats to their use. As a result, the development of novel vaccines or antiviral agents has great value. However, for this to occur there is a need to better understand the process of viral replication, particularly the formation of the FMDV RNA replication complex.

Previously, it has been shown that the FMDV RNA-dependent RNA polymerase 3D^{pol} is able to form higher-order fibrillar structures. Poliovirus 3D^{pol} is also able to form fibrils but for FMDV, fibril production appears to involve RNA. Here, we have defined the conditions that are necessary to form these structures by comparing the effects of mutations in catalytic (DD240/5NN) and non-catalytic (GC216/7AA) domains of the polymerase. It was determined that mutations that abrogated the ability for 3D^{pol} to function also affected the ability to form fibrils in vitro. This study has led to the determination of a novel fibril structure by cryo-electron microscopy (cryo-EM). Docking of the published high-resolution crystal structure (2.75 Å) into the reconstructed cryo-EM density (11 Å) led to the calculation of a pseudo-atomic resolution model revealing a 2-start helical assembly with D_2 dihedral symmetry, assembled from dimers of 3D^{pol}.

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High risk human papillomavirus type 18 interacts with multiple components of the protein kinase A pathway

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High risk human papillomavirus infection is associated with development of epithelial cancers at diverse body sites. To achieve this, the virus remodels various host pathways for completion of the virus life cycle, and this targeting is important for productive infection. Several important features of the HPV life cycle are regulated by protein kinase A signalling (PKA), and changes in this pathway may be important for carcinogenesis. Using biophysical sensors, we show that HPV manipulates PKA activity by regulating cellular responses to elevated cyclic AMP. Interestingly, this feature is lost upon viral integration into the host genome and so may be important for episomal DNA maintenance. To address how HPV can manipulate the PKA pathway, we screened for potential HPV-PKA interaction partners and identified a novel interaction between HPV18 E6 and AKAP95/AKAP8, a nuclear PKA anchoring protein important for regulating chromosomal structure during mitosis and initiation of DNA replication. Using reciprocal GST pull down assays, we have confirmed this interaction. We also show that E6 targeting of AKAP95 does not result in its degradation nor mislocalisation during the cell cycle. Instead, we speculate that E6 targeting of AKAP95 may regulate its phosphostatus by targeting PKA.

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Dynamics of bluetongue virus within Culicoides midges

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Culicoides sp. midges (Diptera: Ceratopogonidae) transmit several viruses of economic and medical importance, including bluetongue virus (BTV), a double-stranded RNA virus within the genus Orbivirus (family: Reoviridae). The distribution of BTV is closely associated with the presence of species of Culicoides midges, capable of propagative transmission. The ability of an arthropod to become infected and biologically transmit an arbovirus is described as its vector competence, which is controlled by the titre of ingested virus and viral strain.

Events during infection of an arthropod vector are very poorly characterised. We develop a quantitative imaging assay for BTV within Culicoides sonorensis tissues. C. sonorensis were fed BTV-1 or BTV-11 and, at various time points following blood meal ingestion, BTV capsid protein and segment 5 mRNA were co-labelled and the percentage of infected cells and infected area within each tissue were calculated as a measure of BTV infection. BTV was detected in the epithelium, fat tissues, midgut, compound eye and brain of infected individuals. BTV was undetectable in the salivary gland, thoracic muscle and oocytes. BTV-1 was detected in a single region of the midgut and unable to infect villi however BTV-11 replicated in villi and a larger region of the gut, which may underlie the increased infection rate. After ingesting a lower dose of BTV-1, segment 5 mRNA expression was greater in infected tissues, indicating suppression of BTV replication following ingestion of a high BTV dose in infected tissues. Data provides unique insight into replication of an arbovirus within an arthropod vector.

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Exploring the interactions of Epstein-Barr virus factor Zta with the human genome.

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The first gamma herpesvirus discovered and sequenced, Epstein-Barr virus (EBV) infects 90% of the population worldwide. It is associated with diseases that include Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma, and many others. Its life cycle can be divided into latency and lytic cycle. When in latency, the viral genetic expression is limited and refrains from producing new viruses, however, this latency can be switched to a lytic behaviour in response to certain signals, resulting in activation of the expression of genes related with viral production. Playing a central role in switching from latency to lytic activity, is viral transcription factor Zta. It dimerizes and interacts with either viral or host DNA sequences that contain Zta response elements (ZREs) and upregulates the level of gene expression. When the interactions between Zta and the human genome were mapped through ChIP-Seq, a new and interesting array of information became available to further investigate the behaviour of Zta and its influence on the host. The use of computational methods for the analysis of the binding sites opened up the door to many different types of explorations. The composition of the sequences where Zta binds to, was scrutinized to expand beyond the canonical ZREs; the context of sequences were moved into artificial reporters to test its enhancing properties; the influence of Zta interactions to distant genes were probed to test if looping events bring distal regions close to promoters; and also the regulation of expression of gene clusters was explored.

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Investigating the significance of internal AUG initiation events in segment 2 of influenza A virus

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In recent years numerous instances of non-canonical translation events producing additional polypeptides from the influenza A virus (IAV) genome have been discovered . Some have noteworthy roles within the virus life cycle, while the function of others remains obscure. IAV segment 2 is known to produce PB1, PB1-F2 and PB1-N40 proteins from AUG codons 1, 4 and 5 respectively. Here, we define expression of two additional PB1-related polypeptides from AUG codons 10 and 11. These methionine codons are highly conserved and direct the translation of N-terminally truncated versions of the primary polymerase product: PB1-N92 and -N111. Using a reverse genetics system , we introduced single mutations of AUGs 10 and 11 (PB1 codons 92 and 111 from methionines to valines) , and these resulted in wild-type-like replication kinetics. However, double mutation of AUGs 10 and 11 severely attenuated A/PR/8/34 virus fitness, despite the mutant PB1 polypeptide displaying normal transcriptional activity in minireplicon assays. Individual AUG 10 and 11 mutant viruses induce elevated levels of type I interferon when compared to wild-type virus. Currently, our data do not distinguish between the hypotheses that PB1 methionines 92 and 111 are important for normal PB1 function or that the shorter PB1 polypeptides play a role in antagonising innate immunity.

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Identification of G-quadruplexes in positive sense RNA viruses

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G-quadruplexes are a non-canonical nucleic acid secondary structure that can form from guanine rich sequences. Recent evidence suggests that RNA G-quadruplexes play crucial roles in the regulation of translation, both at the level of initiation and elongation.

Using a primer extension inhibition assay, a screening system was designed to examine viral RNA sequences that have potential to form G-quadruplexes. This approach relies upon the cation dependency of G-quadruplex formation, which enables such structures to be distinguished from other RNA secondary structures. Sequences of interest are cloned into a plasmid based screening construct, and these sequences can then be analysed for G-quadruplex formation.

Initial screening for putative G-quadruplex forming sequences was carried out upon murine norovirus (MNV), a surrogate for human norovirus. Using a bioinformatics approach, several G-quadruplex candidate sequences were identified and tested in the screening system. Additionally, various G-quadruplex stabilising drugs are available and the ability of these drugs to inhibit MNV replication was examined.

G-quadruplex structures have been identified in a variety of different viruses, including Ebola and Zika virus. As translation is a fundamental process during RNA virus infection, our ability to exploit such RNA structures therapeutically is currently limited by our knowledge of how they act. Such understanding could lead to new antiviral therapies.

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Interactome of MERS-CoV ORF5 protein indicates mitochondrial involvement in viral replication

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The novel, zoonotic coronavirus MERS-CoV, is a positive, single-stranded RNA virus. MERS-CoV has been responsible for outbreaks in 27 countries since its discovery in 2012, with the most recent cases reported in Saudi Arabia. Some cases manifest in severe respiratory distress, which result fatal in about 36% of cases. This highlights the need to deepen the understanding of viral replication and virus-host interactions. This study has generated stable cell lines expressing FLAG-tagged MERS-CoV (EMC/2012) ORF5 protein using an inducible Flp recombinase system in HEK293 cells. Upon induction with doxycycline, viral protein ORF5 was expressed and the resulting interactome was isolated by indirect-immunoprecipitation via the FLAG tag. The identification and quantification of the isolated interactome was carried out using Tandem Mass Tagging (TMT) and LC/MS (Orbitrap) using the SEQUEST search in Proteome discoverer to identify interacting host proteins. We have shown the ORF5 viral protein expression is tightly controlled by the presence/absence of doxycycline. In addition, the induction causes expression of a protein of the correct size for ORF5 in our stable cell line. Furthermore, ORF5 immunoprecipication identified common host binders, which were characterised by a 2-fold increase with respect to controls. Common presence of ornithine aminotransferase (OAT), ATP synthase subunit alpha, stress-70 and heat-shock cognate 71kDa indicate intricate host mitochondrial interactions with ORF5. This suggests that ORF5 may trigger a cellular stress response, which could potentially influence mitochondrial apoptosis or viral immune evasion.

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Oncogenicity in Human Papillomavirus (HPV) correlates with the GC content of viral genes

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Background

Human Papillomavirus (HPV) causes a range of manifestations from benign warts to cancers. Striking differences in the pathogenicity of high risk (HR- that cause cancers) and low risk (LR- that cause benign lesions) HPVs need further investigation to gain insight into risk. The GC content of HPV genomes and its associated differential codon usage may explain the different outcome of HR- and LR-HPVs infections.

Methods

The GC content of 8 HPV genes (E1, E2, E4, E5, E6, E7, L1, L2) was evaluated in 12 HR- and 12 LR-HPVs, selected according to International Agency for Research on Cancer risk classification. For each gene, the GC contents of the HR- and LR-HPVs were determined and statistically compared by T-test.

Results

The GC content varied considerably between the 8 HPV genes, and was found to be statistically significantly higher in LR-HPVs in comparison to HR-HPVs in E1 ($p=0.0009$), E2 ($p<0.0001$), E4 ($p=0.0018$), E6 ($p=0.0068$), E7 ($p=0.0012$), L1 ($p=0.0456$) and L2 ($p=0.0051$), but not in E5 ($p=0.8317$). E4 had the highest GC content in LR- and HR-types (LR-HPVs median=55%, HR-HPVs median=50%) while E6 the lowest (0.4150 ± 0.010 in LR-HPVs, 0.3792 ± 0.006 in HR-HPVs).

Conclusion

LR-HPVs have a higher GC content than HR types, suggesting a differential codon usage pattern, translational efficacy and mRNA stability between the two HPV groups in keratinocytes. Further work will investigate if the lower GC content of HR-HPVs selects for gene expression and viral replication in undifferentiated keratinocytes to explain their role in progression of cancer.

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The Effect of Alphavirus Infection on Cellular Deubiquitylases

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Emerging and re-emerging viral infections are causing great concern across the globe. In recent years Chikungunya virus (CHIKV), a member of the *Alphavirus* family which causes the disease Chikungunya fever, has caused particular concern due to spread of the virus across South East Asia, the Indian Sub-continent, and across the Atlantic to the Caribbean and Central/South America. It is now estimated that CHIKV has been identified in over 40 countries. Currently no vaccines or antivirals are available. Characterisation of how viruses interact with their host cells may help elucidate potential targets for antiviral therapy. The process of reversible ubiquitination, carried out by a large group of enzymes called deubiquitylases (DUBs), plays a key role in the majority of cellular process. Viruses are known to target this system to aid their replication. Using Semliki Forest virus (SFV), a model alphavirus, we have investigated the effect of alphavirus infection on cellular DUB transcript levels in HeLa cells. Out of 36 DUBs tested to date, 8 showed an increase in mRNA levels post SFV infection. All 8 DUBs were also confirmed to increase after CHIKV infection. Experiments are currently underway to investigate if this increase is reflected at the protein level. The work is being extended to investigate DUB expression in fibroblast cells after SFV and CHIKV infection. The DUBs that are elevated following alphavirus infection may reflect novel virus-host interaction mechanisms and could highlight potential targets for anti-viral therapy.

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Probing the control of paramyxovirus transcription and replication by next-generation sequencing

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Title: Probing the control of paramyxovirus transcription and replication by next-generation sequencing

The family *Paramyxoviridae* contains some of the most infectious, pathogenic and economically important viruses known, including human agents such as measles, mumps and parainfluenza viruses. To build knowledge of the viral and host cell factors that control viral transcription and replication and thus contribute to understanding pathogenesis, we have used next-generation sequencing to investigate the transcriptional profiles of selected paramyoviruses. These studies include an analysis of the changes occurring during infection in the relative abundances of (i) viral mRNAs, (ii) read-through mRNAs, (iii) RNA-edited transcripts, (iv) genomic and antigenomic RNAs and (v) defective-interfering genomes. The data obtained have also revealed that a switch-off of viral transcription and replication occurs during the establishment of persistent infections with parainfluenza virus type 5 (strain W3). Finally, the opportunities and challenges of using next-generation sequencing to study viral transcription and replication in paramyxoviruses will be discussed.

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Therapeutic approaches to targeting Epstein-Barr virus related disease

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Epstein-Barr virus infection is associated with several lymphoid and epithelial malignancies. The replication and persistence of the EBV genome in latently infected cells is dependent on the homodimer formation of EBV nuclear antigen 1 (EBNA1) and it's binding to the cognate EBV OriP element. Current chemotherapeutic treatments of EBV-positive malignancies are non-specific to the EBV status of the disease. EBNA1 has been considered as a driver for oncogenesis due to its ability to interact with host proteins whilst evading the immune system. This is possible due to the self-regulatory properties of EBNA1; the full mechanism at which it regulates expression is still unknown. Our work focuses on controlling disease through the inhibition of EBNA1 expression or function. In order to inhibit function, the aim is to disrupt or destabilise EBNA1 homodimer formation. To do this, we have designed and synthesised small peptides, designed to mimic the proline-rich loop of the protein (which is thought to stabilise the dimer) and attached to cell penetrating peptides, to act as competitive inhibitors in the nucleus of infected cells. To inhibit expression we have been investigating agents that interfere with the unique self-regulatory mechanism of EBNA1 expression and their potential as therapeutic drugs. Data will be presented on the effect of these compounds in multiple EBV dependent and independent tumour B cell lines. These peptides and potential expression inhibitors may provide a novel approach to treating EBV positive and dependent disease either by inhibiting viral persistence or affecting the cell survival properties of EBNA1.

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RNA Structure and Function in Emerging

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Nidoviruses are enveloped, positive stranded RNA viruses that have the most complex genetic organization of all plus-strand RNA viruses. Using bioinformatic analysis, the sequence of a new, highly divergent member, of the Nidoviruses has been identified in a pool of intracellular RNA sequences derived from a deep sea metazoan. The virus has been tentatively named Abyssovirus to reflect its origins. The aim of the present project is to validate the virus sequence data by studying the function of several predicted virus sequence features; an unusual translational stop-start signal located in the putative replicase gene and two predicted viral proteases. To do this several gene constructs have been designed and constructed to test the putative functions in each of three background phyla, bacteria (*E.coli),* insects cells and mammalian cells. The translation products of each construct will be described, as will preliminary data on the role of key amino acids, such as those at the predicted protease recognition sites, in the observed functions. Together, this data is consistent with the Abyssovirus being an extant member of the *nidovirales*.

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Association of human papillomavirus type 16 E2 with Rad50-interacting protein 1 enhances viral DNA replication

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Rad50-interacting protein 1 (Rint1) associates with the DNA damage response protein Rad50 during the S phase to G_2/M transition and functions in radiation-induced G_2 checkpoint control. In association with its interacting partner ZW10, Rint1 also participates in subcellular vesicle trafficking. We have isolated a novel interaction between Rint1 and the human papillomavirus 16 (HPV16) transcription and replication factor E2. In this study we demonstrate that endogenous Rint1 complexes with E2 and, while in the absence of E2 Rint1 is localised to the endoplasmic reticulum, nuclear Rint1 localisation is observed in the presence of E2. In some cells Rint1 was found in distinct E2 nuclear foci, predominantly during mid-S phase. Members of the Mre11/Rad50/Nbs1 (MRN) complex were also observed in these nuclear structures, suggesting that localisation of Rint1 to E2 foci may result in recruitment of this DNA damage sensing protein complex. Our results also indicate that E2 does not re-localise ZW10 to the nucleus but efficiently disrupts the Rint1-ZW10 interaction, suggesting that E2 targets the pool of Rint1 protein that functions in sub-cellular trafficking. Interestingly, our data also show that Rint1 expression enhances E2-dependent virus replication and that over-expression of a truncated Rint1 protein that retains the E2-binding domain but not the Rad50 binding domain acts as a dominant negative inhibitor of E2-dependent HPV replication. Overall, our results provide strong evidence for a novel interaction between HPV16 E2 and Rint1 and demonstrate that this interaction plays an important role in HPV replication.

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Utilisation of turnover and translation regulatory RNA-binding proteins during Alphavirus infection

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Post-transcription regulatory control of protein levels in eukaryotic cells occurs at the level of mRNA splicing, export, decay and translation. Turnover and translation regulatory RNA-binding proteins (TTR-RBPs, also termed ARE-RBPs) bind mRNA sequences, often in the 3'UTR, to promote or inhibit degradation of the transcript. One such TTR-RBP, HuR, binds the 3'UTR of the +ssRNA alphaviral genome in both mammalian and arthropod cells. This stabilises the viral RNA and promotes degradation of a sub-set of host mRNA. We assessed the effect of deleting known HuR binding sites from the 3'UTR of the Semliki Forest virus genome by measuring virus replication, production of infectious virus and viability of infected cells. Using RT-qPCR we also investigated whether the role of other TTR-RBPs and specific components of the innate immune system are involved in the HuR mediated enhancement of this viral infection.

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Assessing the significance of host cell shut-off mechanisms in influenza A virus

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Like all viruses, influenza A virus (IAV) co-opts cellular ribosomes to produce viral proteins and, potentially, block the synthesis of cellular antiviral polypeptides. IAV appears to have evolved multiple mechanisms, often virus strain-dependent, to achieve “host cell shut-off”. It is not clear if this reflects robust redundancy, alternative means to the same end, or synergy between mechanisms directed at specific cellular expression pathways.

To answer this question, we focused on viral functions where genetic determinants of the shut-off mechanism have been identified and tested both ‘gain of function’ (GOF) and ‘loss of function’ (LOF) mutations on the background of two viruses, A/PR/8/34 (PR8) and A/Udorn/72 (Udorn) that differ with respect to their shut-off mechanisms. We assessed mutations that modulate PA-X activity, NS1-interactions with CPSF30 or PAF1C, and PB2/PA ability to provoke RNA polymerase II degradation. When effects on overall cellular protein synthesis were considered, we found a hierarchy of contributions in which PA-X > CPSF30 > RNA Pol II loss > PAF1C both with respect to magnitude and time of detectable onset of effect. PA-X and CPSF30 mutations functioned with expected reciprocity (GOF versus LOF) in both virus backgrounds, whereas PB2/PA mutations associated with RNA Pol II degradation acted as LOF mutations in PR8 as well as Udorn. When type 1 interferon induction was considered, the hierarchy was CPSF30 > PA-X > RNA Pol II > PAF1C. Thus not all shut-off mechanisms are equally important in vitro and specific effects on IFN-induction may apply.

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Novel requirement for inverted repeat sequence within EBV IR1 for B cell transformation and EBV replication.

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Deletion of an inverted repeat sequence from within the Epstein-Barr virus (EBV) major internal repeat (IR1) reduced virus production and was essential for B cell transformation by EBV. The inverted repeat is present within each 3kb IR1 repeat and is located partly within the uncharacterised BWRF1 open reading frame. A novel cloning strategy was developed to produce B95-8 EBV BAC viruses with 6 copies of the wild type IR1 repeat, or all copies lacking the inverted repeat sequence, or a revertant of the inverted repeat deletion with all IR1 copies reverted to wild type. Virus producer 293 cell lines were made and the inverted repeat deletion cell lines were found to have reduced virus production. Furthermore the deletion virus was unable to transform B cells into Lymphoblastoid cell lines (LCLs). Although the IR1 region contains the Wp promoter and spliced leader exons for the EBNA mRNAs, normal expression of EBNA-LP was detected during super infection of Raji cells with the inverted repeat deletion virus, implying that the Wp promoter and splicing across the IR1 region still function correctly. The role of the inverted repeat, its contribution to B cell transformation by EBV and the effect of its deletion on the BWRF1 ORF are being investigated.

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Identification of sites of Infectious Bronchitis Virus RNA synthesis

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Infectious bronchitis virus (IBV), an avian gammacoronavirus, is an important pathogen causing significant animal welfare problems and economic losses to the global poultry industry. Positive-strand RNA viruses, including coronaviruses, induce cellular membrane rearrangements during replication forming replication organelles, which are thought to allow efficient viral RNA synthesis. During replication, IBV has been shown to induce the formation of double membrane vesicles (DMVs), zippered ER and tethered vesicles, known as spherules. Although these are proposed to be the site of viral RNA synthesis, this is as yet unconfirmed and this is therefore the main focus of these studies. Historically, dsRNA has been used as a marker for sites of coronavirus RNA synthesis, however IBV-associated dsRNA and nsp12 (the viral RNA-dependent RNA polymerase) do not colocalise in infected cells. We have used bioimaging techniques to elucidate the cellular location of IBV RNA synthesis by investigating the colocalisation of dsRNA and nsp12 with cellular organelles. By comparing the immunofluorescence labelling of cells permeabilised with different detergents, we have demonstrated that both dsRNA and nsp12 can be found within membrane-protected compartments, indicating that the virus could be located in DMVs or spherules, allowing for protection from the host immune response. By incorporating uridine analogues, sites of nascent RNA synthesis have been visualised over the time course of infection with IBV, tracking their colocalisation with viral markers. Using these methods we are able to begin to understand the location of IBV RNA synthesis.

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The many faces of the norovirus protease: how precursors allow spatial and temporal control of protease activity.

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The noroviruses, as many other +ssRNA viruses, generate the proteins responsible for replication of the viral genome through cleavage of a large polyprotein by a virally-encoded protease, to form the viral replication complex (RC). For noroviruses, cleavage of the polyprotein to completion results in the production of six proteins (NS1/2, NS3, NS4, NS5, NS6 (Protease) and NS7 (Polymerase). However, cleavage is not 100% efficient and also varies in efficiency between cleavage sites resulting in the accumulation of stable cleavage intermediates (precursors) to different levels.

In our previous work (Emmott, et. al. 2015, JBC), we described the use of a CFP-YFP FRET sensor, to study the activity of transfected NS6 in a cell-based assay. Here we show NS6 alone cleaved this substrate efficiently, however in the context of viral infection the sensor remained uncleaved. Fully cleaved protease represented only a minority of the protease present during infection, and almost all theoretical protease-containing precursors were detectable, including full-length polyprotein. We generated all 10 potential protease-containing precursors by alanine mutagenesis of polyprotein cleavage sites. All were active on a substrate consisting of polyprotein with its own protease rendered inactive, but only a subset were active on the FRET sensor or a cytoplasmic cellular protein substrate.

In this work we study how sequestration or release of protease precursors to/from the replication complex, either by direct targeting of a precursor to the RC, or interactions between RC components, compartmentalizes protease activity, and may allow for temporal control of stages of the viral life cycle.

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Sas3 and Ada2(Gcn5)-dependent histone H3 acetylation is required for transcription elongation at the *FLO1* gene in the yeast, *Saccharomyces cerevisiae*

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The *S. cerevisiae FLO1* gene encodes a cell wall protein that imparts cell-cell adhesion. *FLO1* transcription is regulated via the antagonistic activities of the Tup1-Cyc8 co-repressor and Swi-Snf co-activator complexes. Tup1-Cyc8 represses transcription through the organisation of strongly positioned, hypoacetylated nucleosomes across gene promoters. Swi-Snf catalyses remodelling of these nucleosomes in a mechanism involving histone acetylation that is poorly understood. Here, we show that *FLO1* de-repression is accompanied by Swi-Snf recruitment, promoter histone eviction, and Sas3 and Ada2(Gcn5)-dependent histone H3K14 acetylation. In the absence of H3K14 acetylation, Swi-Snf recruitment and histone eviction proceed, but transcription is reduced, suggesting these processes, while essential, are not sufficient for de-repression. Further analysis in the absence of H3K14 acetylation reveals RNAP II recruitment at the *FLO1* promoter still occurs, but RNAP II is absent from the gene-coding region, demonstrating Sas3 and Ada2-dependent histone H3 acetylation is required for transcription elongation. Analysis of the transcription kinetics at other genes reveals shared mechanisms coupled to a distinct role for histone H3 acetylation, essential at *FLO1*, downstream of initiation. We propose histone H3 acetylation in the coding region provides rate-limiting control during the transition from initiation to elongation which dictates whether the gene is permissive for transcription.

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Should I stay or should I go? Opal stop codon read-through during Chikungunya virus non-structural polyprotein expression

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Read-through of stop codon signals during translation can result in the production of different virus polyprotein precursors. In this regard, some strains of Chikungunya virus (CHIKV) possess a naturally occurring opal stop codon near the end of the non-structural protein 3 (nsP3) coding region. Read-through at this site results in the expression of all four nsPs, while termination would only allow the production of nsP1, nsP2 and a C-terminally truncated nsP3.

The effect of this naturally occurring opal stop codon on CHIKV genome replication and translation was investigated using both a subgenomic replicon and full length infectious virus. Opal, ochre or amber stop codons were substituted into a dual luciferase reporter CHIKV subgenomic replicon derived from the East Central South African (ECSA) strain of the virus that naturally lacks the opal stop codon. In human cells, replication of the opal mutant was comparable to that of the wildtype CHIKV replicon, while the ochre and amber mutants were unable to replicate; suggestive of specific read-through of the opal stop codon.

Intriguingly, our results suggest that the ochre and amber mutants were able to replicate in either C6/36 Aedes albopictus or C2C12 murine muscle cell lines but exhibited a delay in replication. We are currently investigating whether this delay is a genuine replication phenotype or due to reversion of these mutants to wildtype. Further work into how the presence or absence of an opal stop codon in different isolates of CHIKV influences early replication events is also underway.

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A dileucine motif in the ectodomain of the M2 ion channel protein influences intracellular localisation

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The M2 ion channel of influenza A virus (IAV) is a single-pass type III membrane protein (N-terminus "out", no signal sequence) that plays important roles during both viral entry and egress. Upon entry, M2 facilitates uncoating of the viral particle and during egress, (amongst other functions) can act to equilibrate the pH between the acidic trans Golgi network and the neutral cytoplasm to prevent early conformational flipping of highly pathogenic variants of the viral HA protein. The sequence of the M2 ectodomain is responsible for directing membrane insertion and is highly conserved amongst viral subtypes, making it a candidate for vaccination strategies. However, we have described a rare variant of the viral ion channel, M42, with an altered ectodomain that can functionally replace M2 in the viral lifecycle. M2 and M42 display notably different intracellular localisations, with the former predominantly localising to the plasma membrane and the latter to the Golgi apparatus. Mutagenic analysis of the polypeptides has defined a dileucine motif present in M2 but absent in M42 that underlies this difference. Antibody internalisation experiments suggest that this motif affects retrieval of the ion channel from the plasma membrane. This work defines a new functional motif in the M2 ectodomain that helps explain the functional constraints that underly its conservation.

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Targeting RNA structures in emerging arboviruses

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Emerging arboviruses such as Zika virus (ZIKV) and Chikungunya virus (CHIKV) represent a significant and growing threat to global health. Treatment of arbovirus infections is currently restricted by a lack of specific antiviral therapies and limited knowledge of viral replication requirements.

Structured RNA elements, present in the positive sense RNA genomes of both the flaviviruses and alphaviruses, are known to be essential for virus replication. This study is investigating a range of methods for specifically targeting individual RNA structures within the genomes of ZIKV and CHIKV, during active virus replication.

Following SHAPE mapping of the 5' and 3' untranslated and coding regions of the ZIKV and CHIKV genomes, we are using oligonucleotides incorporating locked nucleic acids (LNAs) to target specific RNA structures. We are also developing RNA aptamers using SELEX to target specific structures and similarly, adhirons using a peptide display scaffold to generate structure specific non-antibody binding proteins.

Utilising these complementary approaches, we aim to target and disrupt the function of individual RNA structural elements, through inhibition of RNA-RNA and RNA-protein interactions required for genome replication. We expect such reagents to be a powerful tool for investigating mechanisms and interactions by which viral RNA elements function within the host cell environment. Furthermore, they will provide a proof of concept for the viability of therapeutic antiviral compounds targeting essential viral RNA structural elements.

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Analyses of transcriptome alterations during human bocavirus (HBoV) infection

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Pseudostratified CuFi-8 cells are the unique non-commercial cell culture system that exclusively supports productive replication of HBoV. We tested commercially available CuFi cells for their capacity to replicate HBoV. Surprisingly despite morphological changes on the glycocalix of infected cells, CuFi-1 and -5 did not support productive viral replication.

The aim was to analyze the transcriptome alterations of CuFi-8 cells during HBoV infection and to compare the CuFi-8 transcriptome to the CuFi-1 and -5 ones in order to identify putative host factors or pathways that enable HBoV replication in CuFi-8 but disable it in CuFi-1 and -5.

Pseudostratified naive CuFi-1, -5, and -8 cultures as well as infected CuFi-8 cultures were harvested for total RNA preparation. A whole transcriptome sequencing NGS approach (MWG Eurofins) on an Illumina platform followed by statistical analyses was performed.

In HBoV infected CuFi-8 cells a total number of 208 transcripts are significantly regulated, of those 95 downregulated and 113 upregulated. Among these transcripts several are known to play a role in fibrogenesis and cancer development, such as Plasma-Protease-C-Inhibitor, ANKLE1, HSP70-2 or NEAT-1. The comparison of CuFi-8 to CuFi-1 and -5 transcriptomes reveal 7 transcripts uniquely expressed in CuFi-8, and 1604 transcripts that are equally expressed in CuFi-1 and -5 but different in CuFi-8.

Our data give rise to the hypothesis that the HBoV infection induces fibrogenesis and triggers cancer development. Moreover, there is an increased likelihood that host factors supporting productive HBoV replication in the infected cell can be identified from the 1604 regulated genes.

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Autophagy impairment by African swine fever virus

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African swine fever virus (ASFV) causes a lethal haemorrhagic disease of domestic pigs. An outbreak in Russia in 2007 has since spread to Eastern Europe and the Baltics and now threatens central Europe. There is currently no vaccine available, however infection with attenuated strains of ASFV can protect against infection with closely related virulent strains. Autophagy is a conserved, essential cell process that regulates multiple pathways that are critical for mounting an effective immune response. Experiments have shown that inhibiting the ability of viruses to regulate autophagy can lead to enhanced immune responses. We have shown that ASFV does not require autophagy for replication and that ASFV can inhibit autophagosome formation, a key step in the autophagy pathway. Through analysis of key proteins upstream of autophagosome formation, we have shown that mTOR (mammalian target of rapamycin), a master autophagy regulator, remains active during infection. Exogenous expression of the ASFV A179L gene has been shown to inhibit autophagosome formation, however we have shown that this gene is not required for viral inhibition of the autophagy pathway. Therefore, maintaining active mTOR during infection may represent an additional means of autophagy inhibition that is exploited by the virus. We have investigated whether autophagy can be stimulated in cells infected with an A179L deletion mutant in a step downstream of mTOR. We predict that a low virulent ASFV strain with an altered ability to modulate autophagy will provide enhanced immunity against virulent isolates.

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Mapping amino acids in huANP32 proteins required for influenza virus polymerase activity

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The human protein ANP32A is co-opted by influenza virus to support its replication (Long et al. 2016), but the mechanism by which it does so is as yet unknown. Replication of the viral negative-sense genome (the vRNA) occurs in two stages. First, a complementary replication intermediate (cRNA) is produced by the resident polymerase, i.e. the polymerase already associated with the incoming vRNA when the virus infects a cell. For this the vRNA genome acts as a template. Next, novel vRNA copies are synthesised from the cRNA for secondary transcription and virion production. For this step, a secondary polymerase, which needn't be catalytically active, is required (York et al. 2013). In vitro studies have suggested that ANP32A, and also its close relative ANP32B, facilitate cRNA-to-vRNA replication (Suguiyama et al. 2015).

In order to investigate the role of ANP32 proteins in influenza virus replication I am generating a set of null human cell lines (HEK 293T) lacking ANP32A, -B, or both. I am using these cells in an in situ polymerase assay to find out what amino acids in the ANP32 proteins are important for supporting influenza replication. I am also asking at what stage of the replication cycle these proteins are required.

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Transcriptional slippage of picornaviral-like RNA polymerases

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Positive-sense RNA viruses mutate and evolve rapidly, in part due to the low fidelity of the viral RNA dependent RNA polymerase (RdRp). Although the substitution rates of such viral polymerases have been studied extensively, less is known about the tendency of these enzymes to "slip" during transcription and produce progeny RNAs with nucleotide insertions or deletions. We recently described the utilisation of programmed transcriptional slippage for gene expression within the *Potyviridae* family (Olspert *et al. 2015,* 2016), raising the question of whether this phenomenon is similarly exploited in other positive-sense RNA viruses.

Potyviruses have a picornavirus-like RdRp. To more accurately assess the sequence requirements for efficient transcriptional slippage in picorna-like viruses, we have inserted the potyvirus slip-prone nucleotide sequence into an infectious clone of Theiler's murine encephalomyelitis virus (TMEV) such that the encoded peptide is cleaved out of the polyprotein, allowing for mutagenesis without affecting viral proteins. We are also investigating the effects of non-programmed aberrant slippage upon the host immune response. This research contributes to our understanding of the mechanisms that shape RNA virus genomic diversity.

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Regulation of the B cell receptor pathway by Epstein-Barr virus nuclear antigens

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Epstein-Barr virus preferentially infects B-lymphocytes and is associated with the development of Burkitt's, Hodgkin's and post-transplant lymphoma. The EBV transcription factors, EBNA2, 3A, 3B and 3C drive immortalisation through the epigenetic reprogramming of cellular genes. Pathway analysis has shown that the B-cell receptor (BCR) signalling pathway is significantly enriched for EBNA-bound genes, implicating EBNAs in its regulation. Deregulation of the B-cell receptor signalling pathway affects cell growth and survival. We have identified EBNA binding sites at promoter proximal and distal elements near the BCR genes CD79A and CD79B and we have confirmed that these genes are repressed by EBNA2 and EBNA3 proteins. Gene expression analysis of further potential EBNA targets in the BCR signalling pathway identified repression of the downstream nuclear factor of activated T-cells genes NFATC1 and NFATC2 by EBNA3 proteins. Western blot analysis confirmed EBNA3B and 3C as negative regulators of NFATC1 and NFATC2 in Burkitt's lymphoma cell lines. These NFATs act as transcription factors that can activate both tumour suppressive and promoting pathways and downregulation may be a tactic used by EBV to increase survival outcomes. EBNA3B and 3C target multiple long-range regulatory regions around these genes and we are using chromatin conformation capture to examine the effects of EBV on the association of these elements with the NFATC1 and NFATC2 promoters. We are also investigating the physiological effects of the EBNAs on BCR signalling.

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Effects of HIV-1 protease cleavage site mutations on incorporation of Gag-Pol into virus particles

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Background: The HIV-1 structural protein Gag precursor Pr55gag can self-assemble into virus particles. Viral enzymes essential for HIV-1 replications include protease (PR), reverse transcriptase (RT) and integrase (IN), which are encoded by pol. The Pol is initially translated as a Pr160gag-pol fusion protein. During or after virus budding, the PR embedded in Gag-Pol is activated to mediate cleavage of Pr55gag and Gag-Pol. It is thought that Gag-Pol dimerization triggers PR activation. Given Gag-Pol is packaged into virus particle via interaction with Pr55gag, it is likely that premature Gag-Pol autocleavage prior to being incorporated into virus particles may in part contribute to a decrease in virus-associated Gag-Pol. To test this possibility, we introduced mutations into HIV-1 that inhibited PR cleavage sites at Gag/Gag-Pol.

Methods: The PR cleavage site mutations created by *PCR-mediated* site-directed *mutagenesis* were introduced into an *env*-deleted HIV-1 proviral vector. Each of the constructs was transiently expressed in 293T cells, and virus assembly and processing were analyzed by Western blot.

Results: Analyses indicate that mutations blocking the PR cleavage sites significantly affect Gag cleavage efficiency but exert marginal effects on Gag-Pol association with Pr55gag. Enhanced Gag-Pol dimerization leads to marked decrease in virus-associated Gag-Pol levels. The level of virus-associated Gag-Pol largely correlated with the level of virus yields.

Conclusion: Blocking the PR cleavage sites has no major impacts on Gag-Pol viral incorporation. Our data support the proposal that HIV-1 PR is triggered to mediate Gag and Gag-Pol processing during or immediately following Gag-Pol incorporation into virus particles.

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The importance of structures in the 5' UTR of FMDV

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Foot-and-mouth disease virus (FMDV) is a single-stranded positive-sense RNA virus in the picornavirus family. It is the causative agent of foot-and-mouth disease which affects cloven-hoofed animals, with outbreaks having a serious impact on economies throughout the world. The 5' untranslated region (5' UTR) of FMDV is unique amongst picornaviruses, due to both its unusually large size (~1,200 nucleotides) and the number of highly structured elements it contains. These include a 360 nucleotide region at the extreme 5' end named the S fragment, which is predicted to fold into a single large stem loop, a poly(C) tract of varying length and a series of 2-4 tandemly repeated pseudoknots. Although the precise roles of these structural elements are unknown, the observation that they are conserved features found in all isolates of FMDV suggests that they are of critical importance to the virus. By using defined mutagenesis of FMDV replicons and virus we have shown that some of these structures appear to be redundant for replication of both virus and replicon, while others are essential. We are pursuing further mutagenesis studies combined with structural analyses to gain a better understanding of the unique features of the 5' UTR of FMDV and use this information to develop novel attenuated vaccines in the future.

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Epstein-Barr virus lytic cycle host and viral proteome and Zta interactors by Mass Spectrometry

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EBV is a member of the human Herpesvirus family that represents a serious health issue and also a significant risk factor for many tumors. EBV has two life cycles, latency and lytic cycle. After the initial infection of epithelial cells, they will establish life-long viral latency in B cells. Following the initiation of EBV lytic replication triggered by many factors, each cell produces hundreds of copies of viral genomes associated with the expression of around 80 viral genes. The EBV origin binding protein termed Zta is encoded by the viral BZLF1 gene. It's a key protein that can disrupt latency, form the nucleus of the viral replisome, assemble the viral replication proteins and target them to the origin of lytic replication, OriLyt.

Our present work is to analyze EBV replisome proteomics changing after switching from latency to lytic cycle and find interactors of Zta using mass spectrometry. HEK-293-ZKO cell line has been used for the study, which is a good model for researching EBV lytic replication in epithelial cells. SILAC labeling was utilized in conjunction with Mass Spectrometry for quantitative proteomic analysis. At last, we identify 60 viral proteins in EBV lytic cycle and a slightly changes in the human proteome . We also see post-translational modifications in both EBV and host proteins. After this global analysis, we are applying chromatin precipitation (ChIP) coupled with mass spectrometry to find Zta interactors at protein level, which is a promising approach to find new therapeutic targets for EBV lytic replication.

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Dual roles for the promyelocytic leukaemia (PML) protein in the regulation of intrinsic and innate immunity to Herpes Simplex Virus-1 (HSV-1) infection

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Intrinsic and innate immunity play crucial roles in the intracellular restriction of many viruses. Intrinsic immunity to HSV-1 infection is conferred by core constituent proteins that reside within PML-nuclear bodies that cooperate to mediate viral genome silencing. This intrinsic antiviral response is antagonised by the HSV-1 ubiquitin ligase ICP0, which targets PML for proteasome-dependent degradation. Recently, an emerging role for PML in the regulation of innate immunity to cytomegalovirus infection has been revealed. Here we investigated the role PML plays in the regulation of innate immunity to HSV-1 infection. We demonstrated that ICP0-null mutant HSV-1 infection efficiently stimulated the induction of interferon-stimulated genes (ISGs) in a PML-dependent manner, a phenotype that required the onset of viral DNA replication. Inhibition of Janus-Associated Kinase (JAK) activity, using the small molecule inhibitor ruxolitinib, also blocked the induction of ISGs in a dose-dependent manner. Unlike PML depletion, however, ruxolitinib treatment of infected cell monolayers did not affect the relative plaque formation efficiency of a HSV-1 ICP0-null mutant, but instead led to significantly enhanced virus yields. These data demonstrate that the PML-dependent induction of ISGs does not directly contribute to the plaque-forming deficiency of a HSV-1 ICP0-null mutant, but rather constricts subsequent rounds of replication. Collectively, our data identifies dual roles for PML in the regulation of intrinsic and innate antiviral immunity to HSV-1 infection, mechanisms that are counteracted by the degradation of PML by ICP0. Our study supports a conserved role for PML in the regulation of innate immunity to DNA virus infection.

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Non-synonymous variants of human interferon lambda 4 (IFNλ4) affect its antiviral activity

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The biological consequences of natural genetic variation in host immune genes remains poorly understood. Here, we investigated the functional potential of non synonymous diversity in human IFNλ4, an antiviral cytokine. Loss of IFNλ4 expression in humans is associated with outcome of infection for many viruses, most notably hepatitis C virus, and has evolved under positive selection in some populations. Using 1000 genomes project data, we identify fifteen mutations that differ in their frequency and geography - twelve never previously described - and thereby establish a panel of mutant IFNλ4 expression plasmids to measure protein production, post-translational modification and antiviral activity. Whereas most variants gave no or modest changes to activity, three caused significant alterations in IFN-stimulated gene expression and antiviral activity against a range of viruses. Changes in activity did not correlate with differences in production or secretion of IFNλ4. Structural modelling placed the variable residues in close apposition, nearby receptor binding helices. From evolutionary analysis, variants occurred at conserved positions in IFNλ4 and other IFNλs. Variants are found at high frequencies globally, while others are restricted to specific populations, including basal human populations. Given the relevance of IFNλ4 to viral infections, our data unveil an unappreciated level of functional diversity in innate immune genes that may affect outcome of infection in human populations. To our knowledge, we are the first to explore systematically the functional potential of human interferon gene variation and provide important insight into the biology of interferons, which will form the basis of future mechanistic inquiry.

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Adenovirus infection and gene delivery are both inhibited by sequential complement-mediated virion inactivation and TRIM21 neutralization

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Unavailable

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Preliminary studies in RSV infection of well differentiated primary nasal epithelial cell (WD-PNEC) cultures derived from cystic fibrosis and healthy term infants.

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RSV infection is the major cause of severe lower respiratory tract disease in young infants, including those with cystic fibrosis (CF). We and others previously demonstrated that airway epithelium is the primary target of RSV infection. However, little is known about the impact of RSV infection on CF airway epithelium. To address this, we established well-differentiated primary nasal epithelial cell cultures (WD-PNECs) from recently diagnosed CF infants to study RSV cytopathogenesis in CF airway epithelium. CF WD-PNECs were successfully generated from nasal brushings of 6 infants at CF diagnosis (4-9 weeks of age). These cultures demonstrated good cilia coverage under light microscopy. However, CF WD-PNECs secreted thick dry apical mucous, consistent with *in vivo* observations in CF patients, while mucous secretions from healthy newborn WD-PNECs was considerably more liquid in form. Despite these observed morphological and physiological differences, RSV growth kinetics were similar in WD-PNECs derived from CF infants or normal healthy newborns. Peak virus titres were evident at 72-96 hpi for both sets of cultures. Relative cell sloughing and pro-inflammatory responses following RSV infection in WD-PNEC cultures from both cohorts remain to be determined. Our preliminary data suggest that this model of CF airway epithelium provides an exciting opportunity to elucidate the cytopathogenic, inflammatory and molecular consequences of RSV infection of airway epithelium derived from very young CF infants.

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NEW ROLES FOR CULLIN-RING UBIQUITIN LIGASES IN INNATE IMMUNITY

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Host immune responses require finely-tuned and tightly-regulated activation and deactivation mechanisms to ensure optimal responses. Dysregulation of these responses often results in failure to clear infections, or the development of excessive inflammatory responses leading to chronic inflammation, tissue damage and autoimmune diseases. Ubiquitylation has emerged as a pivotal process in the regulation of signaling pathways and is involved in many, if not all, cellular responses. Innate immunity and inflammation are no exception. Ubiquitin ligases of the Cullin RING (CRL) family have been reported to control the activation and termination of inflammatory responses, specifically regulating the transcription factor NF-κB, which is considered the "central mediator of immune responses".

My project aims to investigate the role of several members of the CRL family as novel negative regulators of immune responses. Members of the CRL families 1 and 2/5 were selected from RNAi screens, cloned and tested in reporter gene assays, protein-protein interaction assays and immunofluorescence experiments. The results indicate that a number of genes are potent inhibitors of inflammatory responses by controlling the NF-κB signaling pathway, as well as type I IFN production, but not IFN sensing. However, the functional roles and potential mechanisms of action for these proteins remain to be determined. This work can shed light on the regulation of host immunity and inflammatory responses, as well as of anti-viral immunity.

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Characterising the role of a novel innate anti-viral protein in RSV infection.

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Background
Respiratory Syncytial Virus (RSV) is a major pathogen of young infants, causing a spectrum of clinical presentations from mild coryza to severe bronchiolitis. Reasons for symptom diversity remain unclear.
Methods
We previously identified lower baseline expression of gene x in RSV-infected well-differentiated primary airway epithelial cell cultures (WD-PAECs) derived from infants with histories of severe versus mild RSV disease. BEAS-2B cells and WD-PAECs were pre-treated with recombinant protein X or anti-X neutralising antibody at 4oC before RSV infection. We also knocked down gene x by siRNA in BEAS-2B cells. WD-PAECs generated using nasal epithelial cells from term and preterm infants at birth were infected with RSV. qRT-PCR, Elisa and quantitative immunofluorescence analysis of gene x and protein X expression, respectively, were performed.
Results
Pre-treatment of BEAS-2B cells or WD-PAECs at 4oC with protein X decreased RSV infection. In contrast, neutralisation of protein X or knockdown of gene x resulted in increased RSV infection. Gene x/protein X expression increased in newborn derived WD-PAECs following RSV infection, with evidence of increased gene x expression in term- versus preterm-derived WD-PAECs. Preliminary immunofluorescence results suggest increased protein X expression in non-infected cells adjacent to RSV-infected cells. This may, in part explain the non-contiguous nature of RSV infection in airway epithelium.
Interpretation
Our data suggest protein X acts as an innate antiviral molecule against RSV. Relative endogenous expression in infant airways may contribute to susceptibility to severe RSV disease. Therefore, protein X may have biomarker potential to identify individuals predisposed to severe RSV disease.

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Inhibition of the innate apoptotic response by Classical swine fever virus N-terminal autoprotease (N^{pro})

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Classical swine fever virus (CSFV) is a highly contagious positive-sense single-stranded RNA (+ssRNA) Pestivirus of pigs and wild boar (*Sus scrofa*) endemic across much of Asia, Central and South America, Africa and parts of Eastern Europe. CSFV causes persistent, chronic and acute disease which can present as a severe haemorrhagic fever with highly virulent strains.

In vitro CSFV has been shown to antagonise the induction of type-I IFN, partly through a function of its N-terminal protease (N^{pro}) which binds IRF3 and targets it for proteasomal degradation. Additionally, N^{pro} antagonises dsRNA-induced apoptosis however the exact mechanism by which this is achieved remains unknown. Current literature suggests this innate apoptotic response may be targeted at both the mitochondria and upstream of caspase-8. Together, the antagonism of these two innate responses is believed to contribute to the observed immunopathology of CSFV.

This project seeks to characterise the mechanistic basis by which N^{pro} antagonises dsRNA-induced apoptosis. To that end, N^{pro}-expressing stable cell lines and lentivirus-transduced cells expressing EGFP-N^{pro} or shRNA against key dsRNA-sensing pathogen recognition receptors (PRRs) MDA5, TLR3 and RIG-I, will be employed in order to explore the pathways N^{pro} might target. Cells expressing EGFP-N^{pro} will also be used to study the localisation of N^{pro} with respect to host cell proteins implicated in dsRNA-induced pro-apoptotic signalling.

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Impact of Influenza A Virus Morphology on Innate Antiviral Immune Activation

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Influenza A Virus (IAV) causes annual epidemics globally and sporadic pandemics, with significant impacts to human health. The virus can display various morphologies, forming widely-studied spherical virions and elongated filamentous virions. The ability of the virus to form filaments is well-conserved throughout the IAV genus, suggesting that the morphology may have advantages in the host, which are lost when grown in a laboratory setting. The differences in morphology have not been shown to have a definitive effect on viral transmissibility or infectivity, and thus the importance of IAV morphology is still unknown.
NFκB is an important mediator of immune responses and is a frequent target for viral-mediated immune inhibition. Like other viruses, IAV has evolved to block NFκB pathway to enhance its replication, survival and the evasion of host immune responses. Using filamentous and spherical IAV viruses, immunofluorescence microscopy and Western blotting we sought to determine the impact of viral morphology on innate antiviral immune activation, with subsequent effects on viral replication and transmission. Here we show that IAV morphology can significantly affect NFkB activation, innate antiviral responses and may further impact viral transmission.

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Characterization of molecular targets of type-I IFN induction by rotavirus NSP1

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Rotavirus is the most common cause of severe dehydrating diarrhoea, with approximately 590,000 gastroenteritis-associated deaths worldwide in children under the age of 5 years. In the first 24 months of life, almost every child has shown an episode of rotavirus A (RVA) infection. The virus infects both humans and animals but it is usually replication restricted in heterologous mammalians host species, but the underlying reasons for these restrictions are not understood.Viral infections are recognised by specific host proteins, Pattern Recognition Receptors (PRR). These sentinels sense specific viral components, Pathogen-Associated Molecular Patterns (PAMPs), stimulating through the production of interferons (IFNs) the expression of interferon stimulated genes (ISGs), establishing an antiviral state.

RV non-structural protein 1 (NSP1) exhibits the greatest sequence variability of any of RV protein. NSP1 subverts IFN activation targeting proteins in the IFN induction and signalling pathways in a strain-dependent manner. It has been reported that the bovine strain UK antagonizes IFN expression inducing the degradation of IRF3 and IRF7. However, porcine NSP1 (OSU) seems to target preferentially β-TrCP and RIG-I to block IFN induction.

The aim of my project is to characterize the differential targets of RV NSP1 from different host species, with respect to components of IFN induction and signalling pathways.

Using a yeast two-hybrid assay I will define these targets and I will assess the potential role of NSP1 in driving their proteasome-mediated degradation.

A later “read out” of my project will be the quantification of the overall effect of NSP1 on the expression of IFNs.

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

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Due to the availability of robust experimental tools, murine norovirus (MNV) has been used for over a decade as a surrogate model for studying the biology of noroviruses. Depending on the strain of MNV used and the genetic background of the host, MNV can cause acute, lethal, or persistent infections in its natural host, namely mice. Therefore, in addition to providing a tool for the study of norovirus biology, MNV provides a robust experimental system to understand viral pathogenesis, as well as the contribution of viral and host factors to viral persistence. The MNV VF1 protein was previously shown to antagonize antiviral innate immune responses and to contribute to viral virulence in a STAT1 -/- mouse model. We have now used MNV3 to further understand the contribution of VF1 to viral persistence in an immunocompetent background. Our studies indicate that VF1 is not required for long-term viral persistence but it does contribute to persistent viral loads. To better define the mechanism by which VF1 functions, we have used a combination of biochemical and reporter-based approaches to identify the pathways regulated by VF1. Our data indicates that VF1 is an outer mitochondrial membrane protein possessing two mitochondrial localization sequences. We have also demonstrated that VF1 antagonizes the activation of IRF3 during MNV infection via an as yet undefined mechanism. Our current work focuses on further defining the molecular basis of the inhibition of IRF3 activation.

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Inhibition of proinflammatory signaling by HIV-1 Vpr is associated with its localization to the nuclear envelope

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The HIV-1 accessory protein, Vpr, is packaged into virions and enters target cells associated with the core, but its role in viral replication remains unclear. It also localizes to the nuclear envelope, a barrier that the virus preintegration complex must transverse while avoiding triggering nucleic acid sensing and an innate immune reaction. Several previous reports suggest that Vpr may modulate inflammatory signaling, but there is lack of mechanistic understanding.

Here we provide evidence that Vpr can antagonize proinflammatory signaling downstream of the nucleic acid sensing adaptors cGAS/STING and RIG-I/MAVS pathways. Activation of IFNB, NFkB and ISG56 luciferase-based reporters is inhibited by wt Vpr overexpression but not by the DCAF1 binding mutant Q65A or P35A. Moreover, infection by wt HIV-1 suppresses signaling by exogenously added 2'3'-GMP-AMP (cGAMP) to host cells, in contrast to the virus lacking Vpr. Furthermore, Vpr blocked nuclear accumulation of NFkB. We tested a panel of Vpr mutants for their capabilities of inhibiting signaling and localizing to the nuclear membrane. The Vpr mutants that presented aberrant localization throughout the cell also were incapable of inhibiting inflammatory signaling. Proteomic studies indicated that Vpr interacts with components of the nuclear pore, particularly Nup358/RANBP2, whose knockdown prevented Vpr nuclear membrane localization.

Overall, these results suggest that Vpr targets a component or components necessary to trigger proinflammatory responses to the virus in its early life cycle and that function is dependent on the Vpr localizing in or at the vicinity of the nuclear pore.

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The calicivirus VPg protein overcomes translation restriction by host IFIT1.

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IFIT proteins are highly expressed as part of the cell-intrinsic immune response following viral infection or stimulation by type I interferon. IFIT1 has been shown to inhibit translation by sequestration of eukaryotic initiation factors or by directly binding to the 5' terminus of foreign RNA, particularly those with non-self cap structures.

Members of the *Caliciviridae* and *Picornaviridae* families of small positive strand RNA viruses have a viral protein, VPg, covalently linked to the 5' end of the genome. The VPg of caliciviruses is much larger than that of picornaviruses and serves as a cap substitute to stimulate translation of the viral polyprotein. Using in vitro systems we show that VPg dependent translation in different caliciviruses is not susceptible to IFIT1-mediated translation inhibition. We are currently confirming these finding using infection studies. These results demonstrate a new mechanism by which the translation inhibition activity of IFIT1 can be overcome by a virus.

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Quantitative analysis of protein turnover during HCMV infection as an innate immune screen

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In a recent systematic proteomic analysis of temporal changes in host and viral proteins throughout the course of productive HCMV infection, we quantified 750 host proteins that were downregulated >3-fold. We have already demonstrated that this data can be used to identify novel natural killer and T-cell ligands, however such approaches are dependent on bioinformatics and may fail to identify novel mechanisms or novel antiviral restriction factors. An alternative approach to identify biologically relevant molecules requires secondary screens.

HCMV characteristically targets certain key immune ligands and receptors for degradation. We developed tandem mass tag-based proteomic screens to identify proteins degraded by the lysosome or proteasome, however the use of MG132 additionally modulated early- and late-expressed viral proteins which may lead to off-target effects. We therefore employed an unbiased pulsed SILAC strategy to directly assess the rate of degradation and synthesis of host and viral proteins during infection. Combination of these approaches enabled shortlisting of candidate molecules for further characterisation.

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Modulation of Tumour Necrosis Factor Receptor 2 by Human Cytomegalovirus.

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US28-induced changes in host factors during human cytomegalovirus latency

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Human cytomegalovirus (HCMV) is a betaherpesvirus which causes lifelong subclinical infection in healthy adults, but significant morbidity and mortality in neonates and immunocompromised individuals. HCMV establishes latency - maintenance of the viral genome but under a limited transcriptional program that does not produce infectious virions - in cells of the early myeloid lineage.
The G-protein coupled receptor US28 is one of a small number of HCMV genes expressed during latency, and its expression is required for the establishment of latency. In order to understand the mechanism underlying this, a whole-cell proteomic screen was conducted in monocytic cells stably expressing wild-type US28 (US28-WT) or a signalling-defective (but correctly folded) US28 mutant (US28-R129A).
In the screen, two Aim2-like receptor (ALR) family proteins, IFI16 and MNDA, were significantly downregulated in the presence of wild type US28. ALR proteins have previously been linked to inflammasome activation and Type I interferon production and, interestingly, we have obtained preliminary data which suggests that the expression of US28 abrogates DNA-stimulated type I interferon production in monocytic cells. Although such a role has been proposed for IFI16 in the context of HCMV lytic infection in fibroblasts, our observations hint at a completely novel role for MNDA and IFI16 in sensing the latent viral genome.

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Determining the contribution of S-palmitoylation to chicken IFITM3-mediated restriction of avian viruses

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Interferon induced transmembrane protein 3 (IFITM3) is an antiviral protein which has been shown to be upregulated by type I interferon (IFN) via the JAK-STAT signalling pathway. Orthologues of IFITM3 have been found in a variety of vertebrate species including chickens. Chicken IFITM3 (chIFITM3) has already been shown to restrict the replication of influenza A virus *in vitro*. IFITM3 undergoes several types of post-translational modifications including S-palmitoylation. S-palmitoylation of three cysteine residues at positions 71,72 and 105 is essential for the ability of mammalian IFITM3 to restrict Influenza A virus replication, however it is not yet known if this is also the case for chIFITM3. The chIFITM protein contains two S-palmitoylated cysteine residues at positions 71 and 72, but lacks the third site. In order to test the function of S-palmitoylation in chIFITM3, we have made three mutants of the chIFITM3 protein: one where cysteine 71 was mutated to an alanine (C71A), another where cysteine 72 was mutated to an alanine (C72A) and a double mutant where both cysteine 71 and 72 were mutated to alanines (C71AC72A). Confocal imaging has been carried out to determine if removing the S-palmitoylation sites alters the location of chIFITM3 in the cell compared to wild-type. These data will be presented, as well as data from current experiments that are aimed at assessing the extent to which the mutants restrict the replication of both the enveloped Avian Influenza Virus and the non-enveloped Infectious Bursal Disease Virus in avian cell culture.

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Utilisation of the SIV/macaque model to elucidate expression of novel endogenous retroviruses and innate immune interactions

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Endogenous retroviruses (ERVs), descendents of retroviruses integrated into host germline cells which have proliferated over millennia, represent ~5% of mammalian genome sequences. Recent research indicates ERVs may impact on innate sensing pathways, with implications for combating autoimmune disease and viral infection. Based upon bioinformatics searches of full-length ORFs in reference rhesus macaque genome sequences, three ERV lineages have been predicted to contain recently integrated loci (designated PcEV, SERV, CERV). Hence, we are interested to explore the hypothesis that endogenous retroelement expression influences thresholds set for the innate response to exogenous viruses.

The potential of each lineage to produce virion RNA, which may be detectable in plasma, have been investigated using ERV lineage-specific qPCRs applied directly to macaque clinical study material, particularly during the acute infection period when the impact on innate responses will be highest. However, only ERV-specific DNA signals were significantly increased in both Mauritian cynomolgus and Indian rhesus macaque species during acute SIV infection when challenged with several wild-type exogenous SIV strains. By comparison, ERV RNA levels were either not or only weakly expressed in plasma.

As acute SIV infection triggers upregulation or release of ERV-specific-DNA but not RNA transcripts in plasma, we are currently investigating localised expression of cell-associated ERV mRNA in conjunction with upregulation of interferon stimulated genes in multiple macaque challenge studies. These data will help unravel the complex nature and dynamic interplay of ERV expression and exogenous retrovirus infection during primary infection and extend our understanding of innate immune interactions.

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Influenza virus interferon antagonist NS1 is secreted in microvesicles

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Host cells mount an immune response to viral infections, which viruses can counteract through a range of mechanisms. For example, influenza viruses encode NS1, a protein which inhibits the interferon pathway early during infection. We recently showed that NS1, previously described as a non-structural protein, is present in virions, but the mechanism of its incorporation is unknown. It is possible that, like other influenza viral proteins, NS1 is incorporated through a selective mechanism. Alternatively, as NS1 is one of the most abundant viral proteins in infected cells it may be incorporated passively into virions during budding. We previously noted similarities between influenza virions and the microvesicles shed by uninfected cells, and so reasoned that if NS1 was incorporated passively into virions it would also be found in microvesicles. Therefore we used haemadsorption and ultracentrifugation to separate virions and microvesicles shed from influenza infected MDBK cells. Using quantitative mass spectrometry we found that NS1 was present in both virions and microvesicles at similar levels, suggesting that it can be passively incorporated in both cases. Furthermore, the discovery of NS1 in microvesicles suggests a novel mechanism by which influenza could use the host cell's secretory pathways to antagonise the innate immune response in neighbouring uninfected cells, thus allowing the virus to spread from cell to cell more efficiently. Our approach has provided further insight as to the extent by which viruses have evolved to counteract the host immune response.

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Antiviral piRNA pathway in the arbovirus vector Aedes aegypti

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Several ARthropod-BOrne viruses (arboviruses) pose a significant risk to human and animal health. Important emerging arboviruses such as dengue virus, Zika virus, chikungunya virus or Rift Valley fever virus are transmitted by mosquitoes to vertebrate hosts. The infection in vertebrate cells is lytic and may result in severe consequences to the host. In contrast, the infection in mosquitoes is non-cytopathic and RNA interference (RNAi) is considered to be the main mechanism that controls the virus infection. The small interfering (si)RNA pathway is presumably the main RNAi pathway that keeps arbovirus infection under control in the vector; however, more recent findings indicated the involvement of another, less characterized, RNA interference (RNAi) mechanism, at least in mosquitoes: Piwi-interacting (pi)RNA pathway. Using the mosquito-borne Semliki Forest virus (SFV) and mosquito Aedes aegypti-derived Aag2 cells, we found that knockdown of Piwi4 enhanced virus infection, but had no effect on piRNA production. In contrast, knockdown of other core piRNA pathway genes resulted in decrease of SFV-specific piRNAs but this had little or no effect on virus replication, which illustrates the complexity of the piRNA pathway-virus interaction. Little is known about the involvement of other proteins in the pathway, their identity and functional importance. Thus, stable Aag2 cell lines expressing tagged siRNA and piRNA pathway proteins have been generated and used in proteomics studies and findings followed up by functional characterization of identified protein partners.

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Chicken Interferon Inducible Transmembrane (IFITM) Proteins Restrict Infection by Avian Influenza A Virus.

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Interferon-inducible transmembrane (IFITM) proteins are host cell derived restriction factors that are both constitutively expressed and upregulated upon viral infection. These proteins act as the cells first line of defence against invading viral pathogens. Mammalian IFITM proteins have been shown to confer antiviral resistance when challenged with a variety of both enveloped and non-enveloped viruses. So far, three mammalian IFITM proteins have been shown to confer antiviral protection, with specific emphasis placed on IFITM3. We have identified the chIFITM locus on Gallus gallus chromosome 5, previously unidentified in the chicken genome. Chicken embryonic fibroblasts (CEFs) and chick kidney (CK) cells challenged with Influenza A virus (IAV) show an upregulation in chIFITM1, 2, 3 and 5. There is differential upregulation of chIFITM expression, and this appears to be dependent on both the strain of virus and the cell type assayed. We have demonstrated successful knock-down of all four chIFITM transcripts using siRNA in DF-1 and CEF cells. This knockdown increases the susceptibility of the cell to infection by Avian Influenza A virus (H9N2). Conversely, transient overexpression of the chIFITM proteins restricts viral replication suggesting that chickens have four functional IFITM proteins; IFITM1, 2, 3 and 5. It is estimated that poultry will be the major global source of meat by 2018 and will account for 46% of meat consumed by 2022. However, Infectious diseases are a continuous threat to poultry industry. Further investigations into the function of chIFITMs could have implications in securing and maintaining global poultry production.

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A systematic approach to identify Interferon Stimulated Genes (ISGs) with antiviral potential against Ebola virus

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Ebola Virus (EBOV) is an enveloped, negative-sense RNA virus of the Filoviridae family. This zoonotic virus is highly pathogenic in primates and sporadically spreads to humans causing severe haemorrhagic fever, as highlighted by the latest West African epidemic. Given the hazardous nature of working with full-length filoviruses, we implemented a recently described reverse genetics system that produces safe, transcription- and replication-competent virus-like particles (trVLP). This system permits the study of EBOV's cell biology, cellular entry, gene expression, assembly and innate immune evasion.

Expression of ISGs results from the production of type I-IFNs as consequence of signalling cascades being triggered through the activation of pattern recognition receptors by non-self-nucleic acids, such as those found in RNA viruses. These ISGs target specific steps of virus life cycle limiting its spread, replication and leading to adaptive immune response activation. EBOV is capable of counteracting host innate antiviral defences, particularly by blocking type I-IFN production and the cellular responses to exogenously added type I-IFNs. Despite this, we observe IFN treatment of target cells inhibits trVLP production implying that many downstream target genes have antiviral activity against EBOV. We have screened through a well-characterised library, approximately 400 human ISGs, using the trVLP system and identified around 30-40 candidates that may directly inhibit stages of EBOV replication.

The validation and characterisation of candidates are now being addressed. Our recent findings present additional insights into the mechanism associated with the innate immune response to EBOV infection, and further refine the understanding of the cellular function of selected ISGs.

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Budding morphology of avian influenza A viruses

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Pleomorphism is a characteristic of influenza A virus (IAV), which can produce spherical particles of ˜100nm in diameter and filaments up to 30µm. Studies on mammalian strains of IAV identified the viral M1/matrix protein as the main determinant of virion shape. Most human strains of IAV produce filaments and it is hypothesised that this facilitates transmission of virions within or from the respiratory tract. It is unclear whether the same mechanisms might apply to avian strains of IAV or indeed, what the predominant budding phenotype of avian viruses is. This project set out to systematically examine the budding morphology of avian IAV and correlate it with M1 sequence. Cells infected with fully avian or 7:1 reassortant viruses (PR8 with avian virus segment 7s) were imaged by confocal microscopy. Most, but not all avian strains produced large bundles of filamentous virions, in a variety of avian and mammalian cell types. Sequence alignments did not reveal clear patterns of M1 sequence polymorphisms associated with virion shape, but mutagenesis of closely related avian strains indicated that nevertheless, single amino acid changes in M1 determine filament production. Thus filamentous budding, controlled by M1, is a widespread characteristic of avian as well as mammalian IAV.

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Membrane interactions in Picornaviruses

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Picornaviruses are a large family of viruses which include human rhinovirus (HRV) and enterovirus 71 (EV71). Picornaviruses enter cells via endocytosis and from the endosome they undergo uncoating and genome release into the cytoplasm, requiring the virus capsid to interact with the endsosomal membrane. These interactions are facilitated by a trigger such as interactions with the receptor or a drop in pH which induce expansion of the capsid and release of the N-terminus of VP1 and VP4. The N-terminus of VP1 is thought to act as a tether between the virus capsid and the membrane, allowing VP4 to interact with the membrane. Previous work with HRV and poliovirus has shown that VP4 induces size selective pores in membranes which could act as channel for the movement of the RNA genome into the cytoplasm. It is not known if there is a conserved mechanism for membrane interactions and pore formation and therefore the ability of other picornaviruses to interact with membranes has been investigated. We have also selected for HRV16 escape mutants using neutralising antibodies specific for the N-terminus of VP1 or VP4. This provides information on which residues are involved in pore formation.

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Flexible and rapid generation of full-length virus chimeras using InFusion cloning enables characterisatoin of patient-isolated HCV clones in cell culture.

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Hepatitis C virus (HCV) is a major global health issue and the new antiviral therapies are beyond the financial reach of millions of infected individuals. Vaccination remains the best way of combating viral infections. Identification of the best vaccine candidates and evaluation of their performance will require appropriate neutralisation assays utilizing diverse HCV isolates. However, the model isolates used for vaccine development are not representative of the highly diverse populations found in, and between, patients. Only 9 reference isolates, 7 of which were recovered from non-human primates, are commonly used in HCV research. Furthermore, there is a particular paucity of glycoprotein clones from genotype 3, which is associated with hepatocellular carcinoma and refractive to pseudotyping.
We have developed a method for quickly inserting patient-derived E1/E2 genes into full-length virus chimeras. This has enabled us to scrutinise the entry and neutralisation phenotypes of a relevant and diverse panel of glycoproteins. We have generated chimeric virus cassettes comprising the JFH-1 UTRs and NS3-5B genes with the core/p7/NS2 genes from genotype 1 to 6 reference strains. These genotype-specific ΔE1/E2 cassettes are linearised and allow rapid introduction of patient-derived glycoproteins by homologous recombination.
Using our system we have produced over 100 infectious chimeric clones with varying infectivity and antibody neutralisation phenotypes.
This expanding panel will enable accurate assessment of vaccines and therapeutic neutralising antibodies using phenotypically diverse patient-derived glycoproteins. This panel will also serve as a tool to study entry and receptor usage.

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The role of herpes simplex virus-1 proteins pUL7 and pUL51 during egress

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Efficient envelopment and egress of herpes simplex virus-1 requires the conserved viral tegument proteins pUL7 and pUL51. Deletion of either UL7 or UL51 homologues in HSV-1 or pseudorabies virus results in a small plaque phenotype and a reduction in viral titre, suggesting roles for these proteins in assembly of infectious virus particles and cell-to-cell spread. An interaction between HSV-1 pUL7 and pUL51 has been reported previously; pUL51 associates with post-Golgi membranes via a palmitoyl anchor and recruits pUL7. Efficient incorporation of pUL7 into mature virions is dependent on pUL51 expression. We have confirmed the pUL7-pUL51 interaction by co-immunoprecipitation of the complex from infected cells. Using purified recombinant proteins we have shown that residues 29-170 of pUL51 mediate a direct interaction with pUL7 and that this interaction is conserved in at least one other herpesvirus, murid herpesvirus-4. Furthermore, we have identified host cell interaction partners for pUL51 by means of a yeast-two-hybrid screen and mass-spectrometry based quantitative proteomics, these hits will be discussed.

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Towards identification of the cell entry receptor for Type 1 Feline Coronavirus

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Background: Feline coronavirus (FCoV) causes a fatal disease known as feline infectious peritonitis in a minority of infected cats. FCoV research has been hampered by our inability to culture the most common strain, Type 1, in vitro, largely because the cell entry receptor is unknown. We aim to use a high-throughput proteomic approach to identify the Type 1 FCoV receptor.

Methods: Chimeric 'bait proteins' encompassing the spike protein of either Type 1 or 2 FCoV and the Fc region of human IgG were designed and produced. The bait proteins were used for immunocytochemistry and immunohistochemistry with feline peripheral blood mononuclear cells, intestinal tissue, lymphoid tissue and, as a control, Crandell feline kidney (CrFK) cells known to carry the receptor for Type 2 FCoV (aminopeptidase N; APN). Lysates from the CrFK cells were used for immunoprecipitation with the Type 1 and 2 bait proteins.

Results: The Type 2 bait protein bound to the surface of CrFK cells in immunocytochemistry, suggesting a specific reaction with APN. CrFK cell immunoprecipitates are currently being analysed by high-throughput mass spectrometry. The Type 1 and 2 bait proteins showed a surface reaction with feline cells of the monocyte/macrophage lineage using immunocytochemistry and immunohistochemistry.

Conclusion: Feline monocytes/macrophages are specifically recognised by the Type 1 FCoV bait protein and can now be used for immunoprecipitation analysis, which has the potential to identify the receptor for Type 1 FCoV, thus advancing this field of research.

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The Role of 25-Hydroxycholesterol in the Norovirus Life-Cycle

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Human norovirus infection is a leading cause of non-bacterial gastroenteritis worldwide. Understanding viral-host interactions can be essential for the control and prevention of viral infection. Previous reports have implicated cholesterol as important for norovirus endocytosis, however, the role of cholesterol and its downstream metabolites in other aspects of the life-cycle has not been entirely elucidated. 25-hydroxycholesterol (25-HC) is a downstream product of the cholesterol metabolism pathway. It has been demonstrated to have an anti-viral effect against a wide range of enveloped viruses including hepatitis C virus, vesicular stomatitis virus and herpes simplex virus (HSV) as well as non-enveloped viruses such as poliovirus, human papillomavirus-16 and human rotavirus.

In this study, we have started to investigate the role of 25-HC in the norovirus life-cycle using murine norovirus (MNV) as a model system in a variety of MNV-susceptible cells lines. Nystatin, a known inhibitor of MNV infection that acts by sequestering cholesterol from the plasma membrane, resulted in a significant reduction in virus titre, as anticipated. However, exposure to sub-cytotoxic concentrations of 25-HC did not significantly decrease virus titre or viral entry. Moreover, other molecules involved in cholesterol metabolism such as 22-(S)-hydroxycholesterol (22-S-HC) also showed no inhibitory effect on MNV titre. To confirm that MNV-susceptible cells were sensitive to 25-HC, HSV was also employed as a control. Consistent with previous studies, 25-HC had an inhibitory effect on HSV replication in MNV-susceptible cells. Together, our data would suggest that 25-HC has no specific inhibitory effect on MNV replication.

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Identifying the role of herpes simplex virus tegument enzymes during infection

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Upon infection, viruses must usurp the cellular machinery while also evading the immune response. Herpesviruses such as herpes simplex virus-1 (HSV-1) are relatively large and structurally complex with the capacity to carry a number of virus-encoded enzymes within their tegument layer of virions. HSV-1 particles contain at least four enzymes that can modulate host cell function: the ubiquitin ligase ICP0, the endoribonuclease vhs, and two protein kinases pUS3 and pUL13. While the functions of these proteins have been studied in the context of de novo synthesis, the specific role of the virion-packaged enzymes has not been demonstrated. Our approach is to generate viruses that package these enzymes into the tegument but cannot express them from the viral genome, and then use these tools to identify proteins that are modified by the tegument-resident enzymes after they are released into the cytoplasm during virus entry. We have successfully generated deletion viruses for each of these enzymes, as well as clonal complementing cell lines to provide expression of each enzyme in trans for incorporation into deletion viruses. We are using these tools to identify the host cell targets and biological roles of these tegument enzymes during early stages of virus infection.

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Investigating the role of pUL21 during herpes simplex virus-1 replication

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Herpes simplex virus-1 (HSV-1) is a structurally complex, enveloped double-stranded DNA virus that is widely spread throughout the world and has been extensively studied for its biological properties. pUL21 is a tegument protein that is highly conserved throughout the *Herpesviridae* and has been shown to interact with the tegument protein pUL16, which itself interacts with pUL11, a membrane associated tegument protein, and the tail of glycoprotein E. These data suggest pUL21 may function in virion assembly although this and other potential roles of pUL21 during HSV-1 replication are not well understood. Our current study aims to investigate the function(s) of pUL21 during HSV life cycle by studying its interaction with both viral and cellular proteins. We have performed interaction screens using immunoprecipitation of pUL21 in SILAC-based mass spectrometry analysis to identify interaction partners of pUL21. Our studies have identified both viral and cellular binding partners, whose interactions and functions during virus infection are currently being examined using a range of deletion viruses as well as inhibition of the activity of candidate cellular proteins. We have also generated monoclonal antibodies specific for pUL21 to investigate its subcellular localisation and co-localisation with interaction partners during infection. Our data suggests pUL21 has several important roles during the life cycle of HSV-1 in host cells.

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Rewriting Nature's ssRNA Viral Assembly Manual

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ssRNA viruses are present in all domains of life, and are one of the most prevalent forms of viral pathogens (Schneemann, 2006). These viruses encapsidate their cognate genome from a myriad of cellular RNAs within a protective coat protein (CP) shell in order to proceed to the next round of infection in a new cell (Stockley et al, 2013). We have recently shown that this assembly specificity is achieved via multiple, sequence degenerate, dispersed sites within the viral genome, capable of binding cognate coat proteins with nanomolar affinity. These sites, or packaging signals (PS), direct packaging of the RNA genome along a defined assembly pathway (Patel et al, 2015). Using the model plant virus, Satellite Tobacco Necrosis Virus (STNV) and a 5' fragment representing ~10% of the genome, we have identified features of a viral RNA that make it a good assembly substrate. These have been transferred to a non-viral, synthetic, RNA sequence that is packaged preferentially forming non-infectious virus-like particles (VLPs). Addition of this synthetic assembly "cassette" to the 5' end of the native viral RNA improves the characteristics of particle assembly.

The potential applications of these synthetic genomes include VLP vaccines and drug/gene delivery vehicles. Currently, production of VLPs does not take into account RNA:CP interactions and as such, VLPs are often unstable and/or display non-native antigenicity. Here we show that by extracting the essential elements of a viral genome it is possible to create a synthetic sequence that is a better assembly substrate than the wild-type genome.

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The M2 cytoplasmic tail interacts with multiple different cellular pathways to affect viral morphogenesis

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The influenza A virus (IAV) is a single stranded, negative sense RNA virus which causes upper respiratory tract infections. Certain strains of IAV are pleomorphic, producing long filamentous infectious virions many times longer than the diameter of classical spherical viruses. As filamentous IAV is recurrently seen in clinical infections, we are focusing on elucidating the mechanisms of viral morphogenesis. The M segment of the viral genome codes for two matrix proteins: M1 and M2. Using co-immunoprecipitation (co-IP) techniques we aim to understand the relationship between cellular viral response pathways, the M2 ion channel protein and viral filament formation. The cytoplasmic tail (CT: 91-97) of M2 is known to interact with the autophagy pathway in infected cells, and our data suggests that the M2-CT may be subjected to post-translational modifications that affect interactions with cellular partners. Using co-IP and Western blotting, the full length M2 has been seen to interact with the several host proteins and mutation of the M2-CT at residue F91 causes a loss of these interactions. The F91A mutation has been classically used to study interactions of M2-CT with the cellular autophagy pathway, as the M2-CT contains a LC3 Interacting Region (LIR) which mediates binding to LC3, causes its redistribution within the cell, affects cellular autophagy responses and alters viral morphology thorough an unknown mechanism. Our results here suggest that the F91A mutation affects M2 interactions with cellular partners, in addition to LC3, that may have further affects on viral replication and IAV morphogenesis.

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Characterisation of the Pre-Fusion Structure of Herpes Simplex Virus-1 Fusion Protein Using Various Cryo-Electron Microscopy Techniques

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Herpes Simplex Virus (HSV) is a large DNA virus that infects approximately 90% of the population. Antivirals cannot clear infection, coupled with the lack of a vaccine, HSV continues to afflict the population. HSV infection commences with entry into the host cell which requires fusion of host and viral membranes. HSV fusion is mediated by the fusion protein, gB, whose post-fusion structure has been solved by X-ray crystallography. However, there is limited information of gB's pre-fusion form. Elucidating this structure will allow the development of novel vaccines or antivirals. To characterise gB's pre-fusion structure, full-length gB was expressed in vesicles and visualised by cryo-electron tomography and subtomogram averaging. These techniques respectively allow 3D imaging of unique objects and molecular resolution of fusion proteins within a vesicle. We show gB has two conformations, a post-fusion conformation and a compact form, putatively the elusive pre-fusion conformation. While a recent gB pre-fusion model proposes that gB fusion loops are pointing away from the viral membrane, our initial results, using gB tagged with fluorescent proteins, suggest that the fusion loops are pointing towards the viral membrane. To characterise pre-fusion gB at high-resolution, we have produced a minimalistic version of gB consisting of domains I (the fusion domain) and II. Using negative stain, ring structures have been observed, suggesting that they are in a pre-fusion-like conformation. In the future, high-resolution cryo-electron microscopy of domains I & II, combined with subtomogram averaging of full-length gB will allow us to solve, unarguably, the pre-fusion structure of gB.

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Generation of lentiviral pseudotypes from the bat-derived H17N10 influenza virus for the study of viral tropism and neutralization

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Bat influenza viruses have recently been identified in South America, prompting various stakeholders to increase their research in this area to determine potential threats of zoonotic spillover. To date, VSV pseudotypes as well as various combinations of reverse genetics have shown that all genes from this virus are functional, but distinct from other influenza A viruses - to which they are genetically similar. We have produced lentiviral pseudotypes bearing H17 and N10 glycoproteins, and used these to successfully transduce various cell lines (MDCK 1, MDCK 2 and RIE1495). We have found that despite sequence differences, the HA stalk-binding antibodies CR9114 and FI6 will efficiently neutralise bat influenza H17N10 pseudotypes, showing that while this protein uses an as yet unidentified receptor in comparison to other HAs, the stalk remains antigenically conserved. We have shown that an N10 expression plasmid can mediate release of heterologous HA-bearing pseudotypes in the absence of another neuraminidase source - indicating that this enzyme retains the ability to facilitate budding of nascent virions. However, no sialidase activity was detected when the same pseudotypes were used in an Enzyme Linked Lectin Assay.

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Production Of Recombinant Avian Astrovirus Virus-like Particles In *E. coli*

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Astroviruses are small, icosahedral (T=3) non-enveloped positive-sense RNA viruses. Avian nephritis virus (ANV), an avian astrovirus, causes diseases such as runting stunting syndrome, chick nephropathy and mortality, resulting in reduced growth rates and economic impacts on the poultry industry. Astroviruses are prevalent globally with no effective vaccines available due to antigenic variability making them difficult to control. New control strategies are urgently needed as increasingly pathogenic avian astroviruses are emerging. Genome encapsidation is a virus specific process that is essential for generation of progeny virions and ultimately transmission. As understanding of astrovirus assembly in general is limited, we sought to establish a system with which to elucidate the capsid assembly mechanism of ANV, which may lead to new antiviral strategies. To do this, the ANV capsid protein was predictively modelled, revealing high tertiary structure homology to the known crystal structure of human astrovirus capsid, despite low sequence homology. The identified capsid core domain of ANV was cloned with a C-terminal 6 histidine tag and expressed in *E. coli*, subsequent recombinant capsid core protein was purified using a Ni-NTA resin. Initially, expressed capsid core did not readily form VLPs, however, adjustments to the buffer composition yielded formation of VLPs. Thus, we have established an expression platform for the capsid protein of ANV as VLPs, which will enable subsequent analysis of the ANV assembly mechanism.

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Morphological evaluation of CuFi-1, CuFi-5 and CuFi-8 cell cultures before and after infection with Human Boca Virus (HBoV)

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Pseudostratified CuFi-1, CuFi-5 and CuFi-8 cells are permissive for HBoV. However, only CuFi-8 supports the replication of HBoV. It is supposed that HBoV infections results in an alteration of the morphology of the extracellular matrix. It was the aim of this study to compare the morphology of the extracellular matrix of HBoV infected and non-infected CuFi-1, CuFi-5 and CuFi-8 cell lines. The cultured cells were fixed in 2.5% glutaraldehyde containing 2% polyvinylpyrrolidone. After fixation they were processed for scanning electron microscopy (SEM) according standard procedures. After sputtering with gold palladium the specimens were investigated with a SEM (Zeiss Sigma, Zeiss Oberkochen, Germany) using 1.5 kV and an in lens detector. Standardized pictures were taken and the morphological structures of the extracellular matrix were characterized according the following definitions: globular, filiform, reticular and vermiform. The non-infected CuFi-1 cultures demonstrated all morphological characteristics of the extracellular matrix. In the non-infected CuFi-5 cells the globular and filiform structures were predominant, seldom filiform and reticular structures were found. Vermiform structures were absent. In in the non-infected CuFi-8 cells globular and reticular structures were predominant. Some filiform structures were found.. The infected CuFi-8 cells showed only globular structures. Filiform structures were completely destroyed. After HBoV infection with HBoV in all cell lines the morphological characteristics of the extracellular matrix is altered and less structured. It could be shown the after HBoV infection the morphology of the extracellular matrix is altered regarding characteristics and structure.

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Insights into Norovirus Entry Through Thermal Characterisation

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Commonly known as the 'winter vomiting bug', noroviruses are the most common cause of acute non-bacterial gastroenteritis worldwide. Outbreaks can be common in enclosed environments such as care homes and hospitals and apply a significant strain to healthcare services. Noroviruses belong to the Caliciviridae family of non-enveloped positive-sense RNA viruses. In comparison to other positive-sense RNA viruses e.g. enteroviruses such as poliovirus (PV), less is known about the norovirus cellular entry pathways and the dynamic changes that occur to the capsid during endocytosis. Understanding viral endocytic pathways and alternative conformations viral capsids can adopt is a proven strategy for the development of new vaccine approaches (e.g. using virus-like particles) and anti-viral agents.
Thermal inactivation of enteroviruses is known to induce an alternative capsid conformation which mimics that observed during viral uncoating and endocytosis. Using the murine norovirus (MNV) model system, we have employed biochemical and biophysical approaches to characterise the thermal stability of the mature norovirus capsid in comparison to PV. These approaches have demonstrated that thermal-inactivation of MNV also generates a novel intact particle with distinct morphology. Biochemical studies demonstrates this inactivated particle still contains RNA and has the same density as infectious particles. However, low resolution structural data implies that inactivated particles have undergone a structural rearrangement and demonstrate a distinct morphology. Such observations suggest multiple structural and antigenic conformations of the norovirus capsids during the viral lifecycle. Work is ongoing to identify structurally-stabilising capsid mutations and define the structural and antigenic properties of inactivated norovirus particles.

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Understanding temporal expression of VACV genes F13L and A26L and its effect on viral replication.

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Vaccinia Virus (VACV) and other large DNA viruses acquire variation via a number of well-defined mechanisms such as mutation rate; internal recombination, gene duplication and deletion. These parameters are viewed in the context of individual genes.

The extent to which this is constrained by temporal regulation is poorly understood. This body of work explores the effect of manipulating the temporal expression of two biochemically and functionally distinct proteins critical for the VACV morphogenic cycle. The two proteins A26 and F13 are surrogate markers for the two infectious forms of VACV, the intracellular mature virion (IMV) and extracellular enveloped virion (EEV). During infection F13 is produced 4 hours post infection (hpi), correlating with the appearance of EEV. Conversely A26 is expressed later at 6 hpi correlating with the formation of IMVs, accounting for 99% of the VACV progeny.

This study explores this shift via altering the temporal regulation of F13 and A26 by swapping their promoter sequences. Recombinant viruses v(pA26)F13mcherry-FLAG and vF13mcherry-FLAG were produced using transient dominant selection. These viruses were generated via complementation of ΔF13L IHD-J parental virus with two F13mcherry alleles under the control of either the A26 or F13 promoter. Synchronization of F13 with A26 in v(pA26)F13mcherry-FLAG resulted in an attenuated phenotype when compared to vF13mCherry-FLAG. This work presents manipulation of temporal regulation as a method to introduce variability in viruses. This approach allows recombinant viruses to be engineered with novel phenotypes to investigate morphogenesis as well as immunomodulatory genes for the production of vaccine vectors.

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Investigation into the tropism and neutralisation of lyssavirus pseudotypes

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Rabies is a neglected tropical disease that is estimated to cause up to 60,000 deaths worldwide. Whilst current vaccines are available, these are expensive and require multiple boosters to be effective. Additionally, antibodies induced by current vaccines show reduced efficacy against other lyssaviruses, particularly those outside phylogroup 1. A rabies glycoprotein sequence with ≈95% average identity to rabies glycoproteins from current vaccine strains has been investigated in the present study for its infectivity and neutralisation profile. Rabies glycoprotein pseudotypes were produced using a murine leukaemia virus backbone and a luciferase reporter gene. A neutralisation assay was performed using heat inactivated human sera collected pre- and post-rabies vaccination. In comparison to an untreated control, rabies pseudotype infectivity was reduced to <20% at a 1 in 100 dilution and <1% at a 1 in 50 dilution post-vaccination, whilst no virus neutralisation was seen when treated with pre-vaccination sera or with a VSV (vesicular stomatitis virus) control. The pseudotypes were also tested with samples of vaccinated mouse sera at a 1 in 100 dilution. Compared to an untreated control, infectivity of pseudotypes treated with samples that had seroconverted showed <0.05% infectivity whilst a sample that did not seroconvert and an unvaccinated control led to approximately 40% and 65% infectivity respectively. There was also no neutralisation seen for a VSV control. These results suggest that the pseudotype system is appropriate for the investigation of rabies virus infectivity and neutralisation. This system can also be extended to encompass additional lyssavirus strains.

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Characterisation of viral genomes by cryo-electron microscopy

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The recent "Resolution Revolution" in the field of cryo-EM is allowing biologists to reveal viral genome features that have been previously hidden, even in high resolution, icosahedrally-averaged X-ray structures. We recently validated a new paradigm for the assembly of ssRNA virions that make use of multiple, dispersed secondary structures across their genomes, each of which presents a cognate coat protein recognition motif. Packaging Signal-mediated (PS) assembly suggests that it provides selective advantages for viruses that utilise them. We are working to obtain asymmetric structures of Turnip Crinkle Virus, the first eukaryotic virus to have the function of one of its RNA PSs identified. The progress of this study will be described. In addition, we have examined viruses that make use of RNA in their assembly. We are investigating the structural role of the RNA pre-genome (pgRNA) in the life-cycle of Hepatitis B Virus (HBV). SELEX and bioinformatics studies have identified multiple, highly conserved sites across the pre-genome that have the expected characteristics of PSs. It appears the putative PSs play sequence specific roles in making in vitro reassembly more efficient. Asymmetric cryo-EM reconstruction of the T=4 HBV virus like particles (VLPs) reveals a complex between these RNA oligos and the core protein shell. It appears that PSs in this system can act as assembly triggers leading to the formation of the assembly initiation complex. We are currently increasing the sizes of these pgRNA fragments, to understand the role(s) of such complexes in the native virion, which will also be presented.

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Generation of influenza pseudotyped lentiviruses for sero-surveillance in dog populations

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Canine Influenza (H3N8) was first reported in Florida, USA in 2004. Initial transmission occurred from horses to greyhounds and then spread through dog populations in many states, where it is now endemic. Canine influenza (H3N2) outbreaks were later reported in South Korea in 2007, and Guangdong, China in 2011. Both appear to have the same avian virus origin and are also endemic. Since the 2009 swine flu pandemic, the possibility of mammal to human transmission is now considered important, and thus sero-surveillance studies within domestic and feral dogs are warranted.

Pseudotyped viruses (PVs) are a safe alternative to study highly pathogenic viruses, and can be used to assess antibody responses and sero-surveillance, even when the acute infection has passed. The PV is a chimera containing the core of one virus (e.g. a lentivirus) displaying surface envelope glycoproteins of the study virus (e.g. influenza HA, NA). Generating influenza PVs involves transfection of producer cells with required genetic components (Scott et al, 2012 JMGM, 6:304).

We investigated whether functional PVs could be produced via five expression plasmid co-transfection: A/canine/Colorado/30604/2006 or A/canine/Guangdong/3/2011 H3 haemagglutinin (HA), HIV gag-pol, firefly luciferase reporter, HAT endoprotease (HA cleavage) and either exogenous Neuraminidase (NA, for particle release) or different subtype NAs; N1-N4, 8 and 9. Titration results (relative reporter luminescence) indicated functional PVs were produced for Colorado HA with all NAs tested, but not for Guangdong. This data and that determining relative NA activity (Enzyme-linked Lectin Assay) and antibody neutralisation on functional PVs will be presented.

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Investigating the role of glycosylation in the spike protein of infectious bronchitis virus

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One of the major threats to the poultry industry worldwide is the Gammacoronavirus infectious bronchitis virus (IBV). The spike (S) protein of IBV is responsible for viral attachment to host cells and membrane fusion. The S protein exhibits a high level of N-linked glycosylation, where sugars are attached to the protein surface via the asparagine residue in the amino acid sequence Asn-X-Ser/Thr. Glycans exist in three forms: high mannose, complex and hybrid-type. The role of glycosylation in many other viral proteins is well characterised however this has not been extensively studied in IBV. It is hypothesised that S protein glycosylation plays a role in a variety of viral processes including entry and egress from host cells and antigenicity. A range of glycosylation inhibitors have been used to assess the effects of removing or inhibiting the formation of different types of glycan on the function of the IBV S protein in cell culture. These inhibitors have been used to assess antibody recognition and infectivity post treatment. Results indicate that recognition of the S protein by monoclonal antibody has been affected by the inhibition the formation of complex glycans on the surface of the protein. However viral titre does not decrease following treatment with mannosidase inhibitors, suggesting that complex glycans are not essential for virus replication. The effects of the inhibitors on virus entry and egress are also being investigated. Targeting glycosylation in the S protein could provide a platform for improved vaccine design and efficacy against IBV.

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A novel role for domain I of the hepatitis C virus non-structural NS5A protein in virus assembly

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NS5A protein of hepatitis C virus (HCV) plays roles throughout its whole lifecycle. It comprises three domains, domain I is highly conserved throughout all HCV isolates and related viruses, exhibits two different dimeric conformations and has been shown to be required for virus RNA replication. In contrast domains II and III play additional roles in virus assembly.
Based on an alignment of the sequences and analysis of the three dimensional structure, we conducted a mutagenic study of 12 conserved and surface exposed residues in domain I within the context of both a JFH-1-derived subgenomic replicon and infectious virus. We showed that 9 of these residues were absolutely required for virus genome replication, while 3 mutations of the remainder exhibited a partial reduction in genome replication. None of the mutations affected polyprotein processing, suggesting that the replication phenotype resulted from disruption of a specific function of NS5A. Biochemical analysis revealed that the wildtype domain I protein could dimerize and bind RNA in vitro, while these abilities were lost by the 3 mutants with a partial replicative phenotype. In addition, analysis of the phenotype of these mutants in the context of infectious virus identified a role of Val67 and Pro145 within domain I in virus assembly. Surprisingly, these mutants still retained the ability to interact with ApoE. Further experiments are underway to investigate the underlying mechanism involved in virus assembly. We propose that as well as a role in virus RNA replication, NS5A domain I has an independent function in virus assembly.

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The Malignant Catarrhal Fever Virus Complex in Bison, Buffalo, and Goats

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Malignant catarrhal fever (MCF) is a fatal disease of ungulates. OvHV-2 is the principle cause of MCF in the UK. However, numerous clinico-pathological cases have been reported to be OvHV-2 negative. We hypothesised that alternative γ-herpesviruses or co-infection might have a role in causing clinical MCF. The aim of this study was to investigate the γ-herpesviruses present in both healthy and OvHV-2-negative MCF cases of bison, buffalo, and goats using pan-herpesvirus PCR and real-time PCR. The results showed detection of seven γ-herpesviruses species: OvHV-2, OvHV-1, BoHV-6, CpHV-2, caprine lymphotropic herpesvirus (LHV), bison LHV, and reindeer γ-herpesvirus. The quantification data illustrated that OvHV-1, BoHV-6, CpHV2, and bison LHV were endemic in healthy bison, (median viral load 20, 124.52, 40.16, 91.25 copies/100ng respectively). In bison with MCF, similar viral loads were detected for CpHV-2 and bison LHV while OvHV-2, OvHV-1, and BoHV6 were not detected. Testing water buffalo revealed one case of CpHV-2-associated MCF with high viral load (12148850 copies/100 ng). In goats, caprine LHV, CpHV2 and BoHV6 were endemic with (median viral load 30.4 copies/100ng) for the latter. Our results show a complex pattern of infection and co-infection with several ruminant γ-herpesvirus species. However, MCF was only associated with a high viral load of OvHV-2 and CpHV-2. These results help inform the diagnosis of MCF in different ruminant species.

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The Structure and Function of Hantavirus Nucleocapsid Protein

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Hantaviruses are negative sense single-stranded RNA viruses that belong to the *Bunyaviridae* family. They are transmitted to humans from rodents and can cause severe haemorrhagic disease with mortality rates reaching up to 40%. Hantaviruses have been identified in rodents in the UK and have caused several serious human infections in the past few years. The UK hantavirus strain is closely related to other haemorrhagic fever causing hantaviruses including Seoul virus (SEOV) and Hantaan virus (HTNV). The hantavirus nucleocapsid protein (NP) is involved in several essential functions within the virus lifecycle including transcription, translation, protection of the viral genome and has also been implicated in host immune evasion. In this study, a range of recombinant hantavirus NP constructs were designed in order to express both the full length NP and NP truncation mutants with oligomerisation domains removed. Temperature and IPTG concentration were optimised for the expression of these constructs in *Escherichia coli*. Recombinant NP was purified as both native and unfolded protein. Final purification steps were carried out using nickel-affinity and cationic exchange chromatography. In addition, live virus was purified using ultracentrifugation techniques and visualised under electron microscopy. Ribonucleoproteins will be extracted from purified live virus and analysed by electron microscopy. These studies will lead to better understanding of hantavirus NP interactions with viral and host RNA, host cell proteins and other NPs. Disruption of essential interactions may lead to reduced or ablated virus replication which has a potential therapeutic impact.

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CD169+ splenic macrophages allow the intracellular replication of S. pneumoniae preceding the onset of bacteraemia

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In an experimental mouse model, pneumococcal bacteraemia was observed to originate from clonal expansion of a single bacterial cell. During an eclipse phase the bacteria were cleared from blood but survived and replicated in the spleen indicating this organ to be the potential source for the subsequent bacteraemic phase.

Confocal microscopy analysis showed that CD169+ metallophilic macrophages are mainly responsible for pneumococcal survival in the spleen. These cells are present in the spleen marginal zone and despite being very efficient in the uptake of pneumococcal cells, they are permissive to pneumococcal intracellular replication. Moreover, a differential treatment of pneumococcal infected mice with an antibiotic capable (erythromycin) or not (ampicillin) to penetrate splenic macrophages, resulted in the development of bacteraemia only in the ampicillin treated animals. This demonstrates the crucial role that intracellular pneumococci have in the establishment of infection. In an attempt to extrapolate these findings to humans, we tested the fate of pneumococcal cells in a porcine spleen perfusion infection model. In agreement with our findings in mice, we observed uptake and rapid intracellular replication of pneumococci within CD169+ macrophages, which are located, as in humans, in the peri-arteriolar and peri-follicular areas of the spleen.

These data introduce a completely novel concept into the pathogenesis of invasive bacterial infections, in particular regarding the events preceding sepsis. The finding that active bacteraemia arises from bacterial replication foci in permissive CD169+ splenic macrophages will provide substantial inputs to optimize and potentially revise treatment and prevention of sepsis in a clinical setting.

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p53 EXPRESSION IN EPSTEIN-BARR VIRUS ASSOCIATED GASTRIC CANCER AND NASOPHARYNGEAL CARCINOMA

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Introduction: TP53 is a tumor suppressor gene frequently mutated in cancer but in Epstein-Barr virus (EBV) associated epithelial malignancies, including nasopharyngeal (NPC) and gastric cancer (EBVaGC), p53 mutations are uncommon. This study aimed to evaluate p53 mRNA expression and accumulation in NPC and EBVaGC tissues and correlate with EBV LMP1 and LMP2a expression.

Methods: p53 mRNA expression and protein accumulation was evaluated in 3 groups of patients: 9 EBVaGC, 24 EBV-negative GC and 10 NPC cases. The expression of p53 mRNA was evaluated by RT-qPCR and its relative quantification was determined using GAPDH mRNA as normalizer. Accumulation of p53, LMP1 and LMP2a expression were assessed by immunohistochemistry (IHC) using monoclonal antibodies (DO-7, NCL-EBV-CS1-4 and 15F9, respectively).

Results: qRT-PCR results revealed a significant decreased expression of TP53 mRNA in EBVaGC ($2^{-\Delta\Delta Ct}$=0.61; p=0.043) when compared with EBV-negative GC. However, IHC for p53 showed its accumulation in all gastric carcinomas. In all NPC cases was also observed the presence of TP53 mRNA and protein accumulation. The expression analysis of LMP1 and LMP2a in EBVaGC and NPC tumors, demonstrated different profiles. LMP1 is expressed in all NPC cases and absent in EBVaGC, while LMP2a is present in 100% and 44.4%, respectively.

Conclusion: p53 accumulation is observed in all cases, however, comparing EBVaGC and EBV-negative GC it was observed a significant decrease of p53 mRNA. These results suggest that viral carcinogenesis interferes with p53 pathway. In addition, NPC and EBVaGC were characterized by different profiles of LMP1 and LMP2a expression suggesting a distinct EBV-mediated carcinogenesis.

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Neuronal Autophagy Inhibits Production of Mature Semliki Forest Virions

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Autophagy is an intracellular process for protein degradation; damaged proteins are degraded in lysosomes to produce amino acids in times of nutrient deprivation. Semliki Forest virus (SFV) is a positive sense ssRNA virus of the alphavirus genus. Autophagy has been reported to have different effects on replication depending on the virus. In non-neuronal cells, autophagy promotes cell survival during Sindbis and Chikungunya infection, and accumulation of autophagosomes occurs in response to SFV infection.
Human neurons were infected with SFV and autophagy proteins were quantified by western blot. SFV infection decreased LC3-II to LC3-I ratio and increased p62/SQSTM showing that autophagy was inhibited by the virus. Autophagy was induced in neurons with a potent mTOR inhibitor prior to virus infection. Virus production was quantified by plaque assay and found to be lower in cells undergoing autophagy. Virus genome quantification by real time RT-PCR in the same neurons showed a 3-fold increase in viral genome after autophagy induction. In non-neuronal cells however, autophagy gene silencing results in a decrease in both extracellular virus and intracellular virus genome.
The results reported here show that SFV inhibits neuronal autophagy. Additionally, induction of autophagy in neurons reduced infectious virus production. While we observed an increase in the intracellular virus genome levels after induction of autophagy; this increase did not result in higher levels of mature virus protein. This suggests that autophagy may have a protective effect during virus infection and may be used as a means of limiting virus replication and production in neurons.

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Adenoviruses in canids and mustelids: epidemiology, pathology and persistent infections

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Adenoviruses are prevalent in many animal populations and some can cause fatal, systemic disease, for example canine adenovirus type 1 (CAV-1), the cause of infectious canine hepatitis in dogs (*Canis lupus familiaris*) and red foxes (*Vulpes vulpes*). In human beings, adenoviruses usually only cause severe disease in immunosuppressed individuals. Our research has demonstrated that CAV-1 is widespread in red foxes in the UK; 64.4% of foxes are seropositive and 18.8% of red foxes have inapparent infections in tissues, in the presence of neutralising antibodies. This suggests red foxes are an important wildlife reservoir of CAV-1 for domestic dogs. We have also detected and sequenced novel adenoviruses from the tissues of healthy mustelids in the UK, including Eurasian otters (*Lutra lutra*) and pine martens (*Martes martes*). In addition, the pathology of CAV-1 in infected red foxes and dogs has been compared using histopathology and immunohistochemistry. CAV-1 is able to infect hepatocytes, renal tubular epithelial cells and renal glomerular cells, among other cell types, in both species. However, CAV-1 is more frequently associated with infection of vascular endothelial cells in the brain in red foxes compared to domestic dogs. We hypothesise that the mechanisms underlying the susceptibility of different cell types differs between foxes and dogs, and that this may influence the persistence and reactivation of CAV-1. The comparative pathology and pathogenesis of adenoviruses in non-primate species can be used as a model to understand how adenoviruses can persist in tissues and reactivate to cause disease in immunosuppressed human beings.

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RL13 and the UL128 locus co-operate in promoting direct cell-to-cell transmission of Human cytomegalovirus.

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Cytomegalovirus (CMV) is a beta herpesvirus with worldwide distribution and is the leading infectious cause of congenital malformation. It is important that CMV research is conducted using virus strains that accurately represent the agent responsible for clinical disease. However, CMV strains present in clinical samples are unable to replicate efficiently in fibroblast culture until mutants have been selected in UL128 locus (UL128L) and RL13. The UL128L encodes components of a pentameric complex present in the virion envelope, and is implicated in tropism. RL13 encodes a hypervariable virion envelope protein but also suppresses virus release from infected cells, and promotes the efficient spread of CMV by direct cell-to-cell transmission. In this study we show that despite limiting virus release, RL13 has no obvious effect on the temporal cascade of HCMV gene expression during productive infection. However both RL13 and the UL128 expressed independently and in cohort promoted direct cell-to-cell spread that was resistant to neutralising antibodies in fibroblasts. Yet RL13 expression in epithelials promoted virus that was sensitive to neutralising antibodies, indicating that RL13 affects the mechanism of cell-cell spread in a cell-type specific manner. RL13 expression reduced cell-free viral titres due to a reduction in both particle numbers, and particle infectivity. However when viruses expressing RL13 were compared to those lacking RL13, there were no differences in intra-cellular particle maturation by electron microscopy. SILAC-IP was used to identify cellular proteins that bound to RL13, revealing multiple proteins that were recruited to the virus assembly compartment by RL13, during productive infection.

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Identification of two sequence polymorphisms within segment 3 which alter shut off activity but do not affect transcriptional activity of the polymerase complex despite lying in a shared domain of PA and PA-X.

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H9N2 avian influenza viruses (AIV) are now widespread in poultry populations worldwide, causing large economic losses. In recent years reassortment events with other AIV strains has led to H9N2 viruses increasing in pathogenicity in poultry. Our experiments have implicated exchange of segment 3 in this phenotype.

To investigate the molecular basis of this, we characterized the activities of the PA (the main gene product of the segment and a subunit of the viral RNA polymerase) and PA-X (an accessory gene product with a role in host cell protein synthesis shut off) polypeptides from pre- and post reassortment strains of virus. Polymerase activity, assessed by a "minireplicon" reporter assay, was similar between the viruses. However, major differences in PA-X-mediated shut off activity were seen, although, these differences did not correlate with year of isolation or pathogenicity. Sequence comparisons followed by point mutagenesis studies identified specific amino acid mutations adjacent to the PA endonuclease active site that control shut-off activity, without affecting transcriptional function. When these mutations were built into viruses end point replication is not affected however, plaque phenotype of the viruses is altered. Thus PA-X activity can be modulated by mutations in both the X-ORF and the PA endonuclease domain via a mechanism that is independent to transcription.

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The detection, prevalence and disease association of novel porcine parvoviruses

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Introduction: The discovery of novel porcine parvoviruses suggests that these structurally simple ssDNA viruses can undergo rapid evolution with substantial genetic divergence to generate new species. Porcine parvovirus 1 (PPV1) is endemic in pig populations and causes reproductive failure. We present data regarding the prevalence and disease association of 3 novel parvoviruses in pigs; ungulate tetraparvovirus 3 (PPV2), ungulate tetraparvovirus 2 (PPV3) and ungulate copiparvovirus 2 (PPV 4).

Method: 783 tissue and sera samples from archival field and post-mortem submissions from across Europe (Nothern Ireland GB, France and Belgium) were tested for the novel parvoviruses. In house primers were applied in SYBR Green PCR and positive samples confirmed on agarose gel before sequencing. Detailed veterinary pathology data was scored for each sample and analysis of variance was used to identify possible association between the presence of these viruses and disease.

Results: The prevalence of PPV2, PPV3 and PPV4 were highest in grower and finisher groups. PPV2, PPV3 and PPV4 were shown to be multisystemic and were detectable in all tissue types with the highest prevalence being PPV2, detected in 19.3% of samples. All 3 novel parvoviruses showed association with enteric and respiratory disease. PPV2 and PPV3 also had an association with porcine dermatitis and nephropathy syndrome (PDNS).

Conclusion: Presence of the viruses in a wide range of sample types suggests a systemic tropism. The role of these novel parvoviruses as primary pathogens in unclear but statistically significant association with a number of pathologies merits further surveillance and continued research.

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Triple-D Targets: The UK-Philippines Dengue Diagnostic and Drug Targets Research Consortium.

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Dengue is the most important arthropod-borne viral disease of humans and endemic in the Philippines. Dengue infection produces a wide spectrum of clinical presentations, but the factors contributing to differential disease severity are not entirely understood. Although most cases of dengue can be managed at home, the lack of a diagnostic test to predict severe disease with hemorrhagic and plasma-leakage complications, forces patients and healthcare providers to seek hospital admissions for "safety purposes", saturating an already overwhelmed healthcare system. Our overall objective, by adopting an integrated approach, is to correlate disease severity with viral sequence variation and changes in the host transcriptome and proteome, using serum samples from dengue patients with well-defined clinical outcomes. We aim to identify candidate biomarkers for developing novel diagnostic platforms to predict progression to severe disease and identify druggable targets for future antiviral therapeutics. In the first phase of the project we analysed 65 and 36 serum samples using RNAseq and quantitative high-throughput mass spectrometry, respectively, from patients with; dengue without warning signs, dengue with warning signs, severe dengue, pyrexia of unknown origin and healthy controls. Using the bespoke bioinformatics software Tanoti, we characterised full length dengue viral genomes by de novo assembly of the RNAseq data. Other viruses co-circulating in the Philippines were identified. The metagenomics data is being used to inform the proteomic analysis, which has identified and quantified >2000 serum proteins. The data is being analysed to identify potential protein biomarkers, that may be predictive of disease severity.

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Is there a Neurological Legacy Following Zika Infection of Adults?

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The Caribbean and Americas have suffered the rapid spread of Zika virus infection through their populations. The long term health consequences for adults infected with this neurotropic flavivirus remain unknown.

To model the pathology of human Zika virus infection Old World and New World non-human primates were sub-cutaneously infected with a Caribbean Zika virus isolate. Following termination of animals during primary viremia or later time points post viral clearance from the blood FFPE sections from multiple tissues and brain regions were analysed for Zika virus RNA (RNAscope) and host responses (immunohistochemistry).

All animals developed detectable blood viremia which was rapidly cleared however low level viremia was detected sporadically for at least 6 weeks. Following 6 weeks infection Zika virus RNA was detected within male (red-bellied tamarins) and female (rhesus) reproductive tissue. Higher levels of viral replication were detected within male tamarin tissues. Zika virus RNA was also detected in tissues associated with biological secretions containing Zika virus during human infection. At 6 weeks post infection Zika virus RNA was present within the brain. Inflammatory astrogliosis (GFAP) and microgliosis (iba-1) were present and, notably, disruption of MAP2 neuronal dendrite staining within occipital lobes.

The presence of virus and host inflammatory responses 6 weeks post infection raises the possibility that, in addition to being neurotoxic in foetuses, Zika virus infection may initiate a chronic inflammation in adult brains. Further work will establish whether the observed neuroinflammation resolves or persists with additional neuropathology developing.

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Rabies Virus Glycoprotein is Trafficked to the Plasma Membrane and Internalises to Intracellular Compartments in a Strain Specific Manner

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Rabies virus (RABV) is a neurotropic virus that results in a devastating disease of the central nervous system (CNS) that is responsible for up to 70,000 deaths annually. Today, approximately 95% of RABV infections occur in rural communities across Asia and Africa, in regions that are not equipped to deal with the impact of the virus.

RABV is a non-segmented negative sense RNA virus classified within the family *Rhabdoviridae*.
The RABV genome encodes five proteins: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and the RNA-dependent RNA polymerase (L). RABV G is the only viral protein exposed on the surface of the virion and mediates RABV interactions with cellular receptors, pH-dependent virus fusion, and promotes viral entry and trans-synaptic spread within the CNS. However, the cellular roles of RABV G during the RABV lifecycle post-virus entry remain to be defined.

Here we identify a novel, strain specific trafficking event for RABV G. Newly synthesised RABV G from the pathogenic Challenge Virus Standard (CVS) strain was found to traffic to the plasma membrane (PM) and undergo clathrin-mediated endocytosis whilst RABV G from the attenuated vaccine strain SAD-B19, showed elevated levels of PM expression, but did not internalise. CVS G internalisation was clathrin dependent and occurred through early/recycling endosomal compartments. We speculate this transport of RABV G dictates its localization during RABV infection and may contribute to the altered pathogenicity of CVS vs SAD-B19 strains.

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Genetic marker associated with influenza A(H5N1) virus adaptation in the mouse model

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Amino acid changes in the polymerase genes of H5N1 influenza viruses may be implicated in the adaptation of avian influenza to a mammalian host. Two mouse adapted (ma) strains, NIBRG-14ma and NIBRG-23ma, which were produced by serial passage in mice, showed increased infectivity and lethality compared to their parent viruses. To help further understand the mechanisms and genetic molecular markers associated with host adaptation of H5N1 influenza viruses, Next Generation Sequencing methods were used to gather consensus and deep sequencing data and in addition, multi-cycle growth curves were performed to investigate the increased pathogenicity in mice. The results showed a single change in the PB2 gene of both ma viruses compared to their parent virus, resulting in a non-synonymous amino acid change of both ma viruses. Enhanced growth rates shown in the ma viruses compared to the parent viruses are likely to be due to increased PB2-dependant polymerase activity.

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IBV accessory protein 4b and stress granules

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Infectious bronchitis (IB) is an avian disease that is responsible for significant economic loss within the poultry industry, leading to reduced egg production and weight loss. The etiological agent; infectious bronchitis virus (IBV) is a single-stranded positive sense RNA virus of the gammacoronavirus family. IBV expresses four known accessory proteins during infection (3a, 3b, 5a, 5b). The role of these proteins is unknown, but it is known they do not play a role in replication. We have confirmed the presence of an extra protein expressed by the pathogenic strain M41, known as 4b. This protein is highly conserved between the strains of IBV and turkey coronavirus (TCoV). This protein is expressed at lower levels due to a non-canonical transcriptional regulatory sequence (TRS) and forms a granular like pattern during infection as seen by immunofluorescence. Mass spectrometry data suggests this protein is involved in translation and/or stress granules. Stress granules are a feature of IBV infection, with the virus able to induce their formation. We have confirmed that during infection, 4b interacts with the ribosomal protein S25, which has been shown to be an important protein for other viruses including HCV. Further work will focus on this protein and its ability to aid viral translation or inhibit/induce the cellular response to stress.

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Biodistribution and viral kinetics of Zika virus infection in Old and New World Non-human Primates

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Zika virus (ZKV) has emerged as a significant global pathogen, although the full spectrum of pathogenesis in susceptible hosts is not fully understood. To gain a better understanding of host range and virus dynamics we have undertaken a series of studies in different non-human primate species, including new and old world monkeys. Initially, virus kinetics and tissue biodistribution were determined by plasma-derived qPCR and in-situ techniques in pairs of Indian rhesus macaques (old world) and red-bellied tamarins (new world).
To further assess distribution and viral burden of ZKV RNA in multiple tissues recovered post-mortem, we employed quantitative RT-PCR, comparing diverse tissue types from peripheral and central anatomical sites. Selective sequence analysis of ZKV recovered during the peak of acute infection is being used to determine the nucleotide composition of the same viral isolate (Puerto Rico/PRVABC59) introduced into independent NHP species, at a time when tissue sequestration and viral dissemination is highest. We are further characterising the early dynamics of ZKV infection by applying PrimeFlow RNA technology to understand the range of cell lineages (major lymphocytic and myeloid) most associated with acute ZKV infection.
Using these combined approaches, we are building a more complete picture of the host range and intra-host dynamics of ZKV infection. Data will be presented on these studies to illustrate the major differences and similarities between ZKV infection in multiple species of non-human primates and the implications for the current ZKV epidemic will be discussed.

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Mixed Signals: The role of Hippo signalling in HPV associated cancers.

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Human papillomavirus (HPV) infections are the main cause of cervical cancer, with high-risk types such as HPV16 and HPV18 accountable for 70% of this cancer type. More recently, HPV infections have also been linked to other cancer types such as head and neck cancer and oropharyngeal cancers. Although transformation activity of high-risk HPV is linked to the interaction between E7 oncoprotein and retinoblastoma family proteins, it does not completely account for HPV's cellular transformation and immortalization activities. Thus, it is reasonable to speculate that other HPV-host interactions may be involved. The Hippo signalling pathway is a key regulator of cell proliferation and differentiation and HPV has been shown to modulate a downstream effector, Yes-associated protein (YAP), in the pathway to induce transcription of a subset of pro-proliferative genes. However, the specifics of how the virus is able to modulate this pathway remain incompletely understood. We show here the first evidence of viral modulation of the core Hippo components; our results highlight that HPV positive cervical carcinoma cells have lower levels of MST1, a key player in the core Hippo pathway, relative to HPV negative cervical cancer cells. In addition, our study indicates that this modulation is at a transcriptional level. Further studies will be directed at identifying the viral oncoprotein responsible for Hippo manipulation and observing the effects of reversing viral manipulation. Understanding how HPV targets Hippo signalling may present novel opportunities for HPV therapeutics, in the form of pathway inhibitors.

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A differentiated human neuroblastoma model of herpes simplex latency

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Herpes simplex viruses (HSV) establish life-long latent infection in neurons, characterised by a general repression of virus gene expression and lack of virus replication. Periodic reactivation of the virus from this state leads to both recurrent disease and transmission to new hosts. Mouse studies from our lab have shown that the HSV latency-associated transcript (an abundant viral RNA produced during latency) aids the long-term survival of the latent cell population, as well as limiting the frequency at which the virus reactivates at the single cell level.

However, to understand (and therapeutically interfere with) the molecular mechanisms governing HSV latency in humans, as well as to discern the strength of hypotheses drawn from animal models, it is necessary to develop a human neuronal model of latency.

In this report we present the development of such as model - applying a differentiation protocol to a human neuroblastoma cell line to produce a homogeneous non-dividing population of bipolar neurons in which latency can be established with both replication-defective and (in the presence of acyclovir and interferon-alpha) replication-competent HSV.

Using reporter viruses expressing β-galactosidase from different promoters, as well as qRT-PCR analyses, we have been able to investigate latency in this model by defining the frequency and kinetics of promoter activation, as well as the total levels of viral gene expression from latent and lytic promoters. Furthermore, this model will allow for investigations requiring a large and homogeneous cell population, such as analysis of the latently infected cell transcriptome by RNAseq.

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The contribution of Epstein-Barr virus towards apoptotic resistance in extra-nodal NK/T cell lymphoma.

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Epstein-Barr virus (EBV) is a γ-herpesvirus that persists asymptomatically in ~90% of adults. Despite its prevalence in healthy people, EBV is associated with a number of malignancies that contribute to 2% of the global cancer burden. One such malignancy is extra-nodal NK/T cell lymphoma (ENKTL). These tumours exhibit high chemoresistance, with patients accordingly having extremely poor prognosis. The rarity of these cancers has limited understanding of the contribution of EBV to pathogenesis, and the mechanisms behind evasion of conventional treatments.

A panel of ENKTL cell lines have been previously established from human tumours to be used as *in vitro* models of this disease. We have investigated the chemosensitivity of the tumour cell lines to conventional chemotherapeutics and to BH3-mimetic drugs, that can trigger the intrinsic apoptotic pathway, either as single agents or in combination. Furthermore, we have assayed the expression of EBV genes and members of the intrinsic apoptotic pathway.

Transcriptional analysis confirmed the expected latency II pattern of viral gene expression associated with ENKTL, but also revealed great diversity in levels of the major viral transformation genes: latent membrane protein 1 (LMP1), LMP2A and LMP2B. Interestingly, LMP1 showed an inverse correlation with expression of the pro-apoptotic BH3-only proteins, BIM and BID. Conventional DNA-damaging drugs, such as etoposide and methotrexate, did not induce apoptosis in ENKTL cell lines. However apoptosis could be induced to varying degrees with BH3-mimetics drugs that target BCL-X_L and MCL-1.

Taken together, these data suggest that BH3-mimetics drugs could be a therapeutic avenue for ENKTL.

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Generation of a recombinant GFP-tagged infectious bronchitis virus (IBV)

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Infectious Bronchitis Virus (IBV) is a highly contagious *Gammacoronavirus* which infects the epithelial surfaces in poultry. IBV has been shown to have the highest direct disease costs for poultry in the UK and even though vaccinations currently exist the disease is prevalent in all countries with a poultry industry. Using reverse genetics system developed at The Pirbright Institute, enhanced green fluorescence protein (eGFP) is being inserted into the pathogenic clone of IBV, M41-K. The reverse genetics system is based on the use of vaccinia virus which is a carrier for the IBV genome in which mutations can be made. eGFP is being inserted to replace two different sequences: gene 4b and open reading frame (ORF) 7. These regions have been chosen as it is thought that their deletion does not have detrimental effects on viral replication and pathogenicity. Recombinant IBVs tagged with split eGFP containing tagged M, 4a and 5a proteins are being generated. The viral proteins will be fused to the 16 C-terminal amino acids of the eGFP. This is a much smaller insertion, which may be better tolerated by the virus. The large transcomplementing GFP fragment will be expressed in chicken cells. The viruses containing the entire eGFP sequence will allow tracking of viral spread within tissues and cell, the concentration of virus present in each location can be determined using a fluorescence reader. The viruses tagged with split eGFP can be used to track when and where a specific protein is expressed within the virus life cycle.

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Establishing the first Cryptosporidium drug screening using the culturing platformC. More, L. Josse, L. Yiangou, E. Saintas, M. Michealis and A. Tsaousis, Laboratory of Molecular and Evolutionary Parasitology, University of Kent, UK

Charlotte More, Lynne Josse, Emily Saintas, Lyto Yiangou, Martin Michealis, Anastasios Tsaousis
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Cryptosporidium is an apicomplexan parasite which causes cryptosporidiosis, a diarrhoeal disease. In immune-competent hosts this is self-limiting however in immune-compromised hosts this is not the case and requires treatment.
The aim of this research is to repurpose a current therapeutic by culturing colo-680 cell line, infecting it with cryptosporidium and investigating the oocyst production and running MTT drug assay screens for damage to host cells without infection for morphology changes and then for effect on the cryptosporidium.
Colo-680 is being used as it maintains a cell line after infection for longer without apoptosis and has up-regulation in fatty acid synthase. The FDA drug list from ChemSellek is being used. If there is a lack of success look at proposed and possible targets and possible compounds using computer aided drug design and the Protein Data Bank.
Current drug treatments are; Nitazoxanide and Artemisinin however the issues with both of these drug compounds is; resistance or the effects of the drug on an already immuno-compromised host. Therefore there is a need for a new therapeutic agent against Cryptosporidium. Currently there is a move to repurpose compounds which are already on the market, it will be investigated whether it is possible to repurpose a current therapeutic.
Assays have been run using the FDA drug list against colo-680 to assess cell viability. Drugs which do not overly affect cell viability have moved on to stage two and been screen for activity against Cryptosporidium.

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Ancient divergence, and recent diversification of peridinin dinoflagellate plastid genomes

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The plastid genomes of peridinin-containing dinoflagellates are highly unusual, retaining only twelve protein-coding genes. Previous studies of peridinin plastid genes have found evidence for unusual evolutionary features, including novel insertions and open reading frames, use of alternative translation initiation codons, and the loss of otherwise conserved residues. However, placing these events in an evolutionary context has been limited by the amount of sequences available, with effectively complete genomes only available for three peridinin dinoflagellate species. We have identified dinoflagellate plastid mRNAs from transcriptome data from NCBI and MMETSP, vastly increasing the number of sequences available. We have used this data to document the evolutionary changes that have occurred in peridinin plastids. The origin of dinoflagellate plastids was accompanied by extremely divergent evolution, specifically underpinned by a change in the selective factors acting on early dinoflagellates. These divergent sequence features have continued to accumulate in individual dinoflagellate lineages. We show that this divergent evolution has been biased throughout dinoflagellate history to photosystem I genes, and the stromal faces of peridinin plastid proteins, suggesting possible evolutionary drivers for the extraordinary events seen in this plastid lineage.

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BIODIVERSITY OF CILIATED PROTOZOA IN SOIL HABITATS AND THE CORRELATION BETWEEN CILIATES AND TRACE METALS

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Soil biota, including protists, have fundamental roles soil in processes, and have been considered as potential indicators to assess soil quality. Ciliates are one of three main groups of protozoa. They are an abundant group of soil micro-organisms, which play important roles in food webs by controlling smaller micro-organisms and recycling organic matter. Soil factors may have an impact on soil quality and may influence the communities of soil ciliates. This research focuses on the investigation and comparison of the diversity of ciliates in two agricultural ecosystems and one natural ecosystem in order to find the correlation between them and trace metals. The results showed that there was no difference in the ciliate species richness between natural and agricultural soil, but there were significant differences in the abundance of ciliates between the three habitats and over time. Ciliate abundance in natural soils was much higher than in agricultural soils for all months observed, in which the highest abundance was shown in July. The concentration of trace metals was also different between habitats and over time. Concentrations of metals were highest in one farm in all months, except for Pb which was highest in the natural soil. Interestingly, there was positive correlation between all trace metals and ciliate abundance, except for Cd. This demonstrated that the low available concentrations of trace metals in study sites were insufficient to have toxic effects on ciliates. On the contrary, ciliates responded positively to trace metal levels.

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APPELS - A Probe of the Perodic Elements for Life in the Sea

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Metals are a fundamental component of life; the biological processing of oxygen, nitrogen, sulphur and carbon all rely on metalloproteins. With an estimated 1 in 3 proteins containing a metal co-factor, but less than half of all metalloproteins being characterised, the wider study of transition metals in the marine environment is warranted. Marine phytoplankton, capable of oxygenic photosynthesis play a major role in the cycling of carbon, nitrogen and phosphorus and act to regulate the global environment and climate. Therefore, deciphering how the ensemble of the core metalloproteins has evolved and how they interact, has important implications for understanding the biogeochemical cycling of elements in the ocean. Within the new APPELS project, we take a novel, multi-faceted approach towards a comprehensive view of the essential elements to life in the ocean. Multi-level HPLC protein fractionation is paired with ICP-MS metallomics for the elucidation of metal containing proteins followed by their identification via LC-MS/MS proteomics. Using this novel methodology, we aim to expand the known biological periodic table by providing insight into the core elemental requirements of phytoplankton both in present day and past environments.

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Determining the minimal selective concentrations of macrolides in complex microbial communities

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Resistance to antibiotics has been described as a global public health crisis and the O'Neill Review estimates that by 2050 antimicrobial resistance will be the leading cause of death, with a predicted 10 million deaths per year.
An underappreciated reservoir of resistant bacteria is present in the environment, particularly those impacted by anthropogenic pollution. There is continuous release of antibiotics from human activity that leads to measurable concentrations in surface waters. Aquatic antibiotic concentrations are generally low relative to those used to treat infection, although higher concentrations can be associated with antibiotic manufacturing. Until recently it was thought that selection for resistance does not occur at environmental antibiotic concentrations. However, research by Gullberg et al. (2011 and 2014) used single species competition assays to determine selection for resistance to some antibiotics occurs at concentrations found in surface waters (ng/L - µg/L).
Macrolide antibiotics are important for treatment of both human and veterinary infections and were recently added to the European Commission Water Framework Directive's priority watchlist as they are frequently found at relatively high concentrations in aquatic environments.
We investigated the selective potential of macrolides using an aquatic complex microbial community in week-long evolution experiments. We were able to determine how macrolides affect a population rather than a single species. QPCR was used to measure the prevalence of resistance genes within the community allowing minimal selective concentrations to be estimated.
Data presented suggests that current environmental macrolide concentrations do not select for widely distributed macrolide resistance genes under laboratory conditions.

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Establishing a novel drug discovery platform for the identification of anti-microbial compounds against the "brain-eating amoeba"

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Naegleria fowleri ("the brain-eating amoeba") is a unicellular amoeboflagellate and an opportunistic parasite to humans, which causes Primary Amoebic Menignoencephalitis (PAM). PAM is an acute disease affecting the central nervous system associated with a case fatality rate > 95%. Presently, there is no effective drug against this disease. Over the past decade there has been a rise of infections of *Naegleria fowleri* in developing countries, thus it is crucial to identify and develop new anti-microbial drugs against this fatal pathogen. We have established a novel screening platform and performed an initial drug-screen by employing a collection of drugs and established anti-microbial agents using the non-pathogenic *Naegleria gruberi* as an easy-to-handle model organism for the discovery and investigation of anti-*Naegleria* therapeutics. The viability of the treated cells was monitored using *in house* established colorimetric assays. By employing a library of 1443 FDA approved drugs and the "Pathogen Box" that contains 400 diverse, drug-like molecules active against neglected diseases, we have a performed a high throughput screening on *N. gruberi.* Current preliminary data indicate an effective response of certain anti-microbial agents against *Naegleria gruberi*, whereas others contradict already published data. The described screening platform will promote the discovery of anti-*Naegleria* drugs, will provide the basis for similar discovery platforms for additional parasitic microbes, and will enable systematic chemical biology approaches designed to decipher *Naegleria*'s biology.

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The antimicrobial activity of *Serratia plymuthica* culture supernatant

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PROJECT HISTORY:

According to a previous study concerning the natural metabolites of seaweed associated marine microorganisms .

A bacterial strain with a characteristic colony morphology comprising of bright orange color demonstrated inhibitory activity against a variety of gram-positive bacterial test strains . Ribotyping analysis results showed that the strain had a sequence identity (99%) to *Serratia plymuthica.*

HYPOTHESIS:

S. plymuthica also produces another inhibitory activity in the culture supernatant. This phenotypic characteristic of the antimicrobial producing strain indicates the presence of more than one compound responsible for antimicrobial activity .

AIM:

The aim of my project is to investigate the production of antimicrobial activity in *S. plymuthica* culture supernatant by combining microbiology based methods with molecular biology techniques.

My project includes optimizing the best growth conditions for the production of antimicrobial activity and studying the effect of these conditions . My project also involves the genetic analysis of *S. plymuthica* and cloning the genomic regions that code for antimicrobial production.

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Comparison of the gastrointestinal resident microbiota in diploid and triploid rainbow trout

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The microbiota forms an integral part of the innate immune defence system of the gastrointestinal (GI) tract, important to stimulate the GI immune system and allow differentiation of food particles and pathogenic and normal bacterial flora. It also provides an effective barrier to infection through competitive exclusion, and in some cases antagonism to potential pathogens through the production of extracellular products. However the role that the individual microbe plays in the health and nutrition of fish is still poorly understood, making investigations of the composition of the intestinal microbiota crucial for future health management strategies for the aquaculture industry. In fish production, sexual maturity diverts a substantial amount of energy to reproduction which can comprise of up to one third of fish body weight, with a corresponding decrease in harvestable product. Triploid rainbow trout (Oncorhynchus mykiss) are increasingly being farmed for food as they are sterile and therefore minimise reproductive-related production loss. To date, no comparison of the effect this has on the GI microbiota and subsequent gut immunity has been performed. We present preliminary characterisation of the intestinal microbiota using a combination of culture based and 16S rDNA analysis including sequence and DGGE-based analysis to determine differences between the microbiota of identically reared triploid and diploid juvenile rainbow trout. Our studies indicate one of the main cultural components is Carnobacterium spp, which has been linked as normal GI flora in other fish species and also associated with spoilage in farmed fish production.

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Emiliania huxleyi and associated bacterial response to oil pollution under projected atmospheric CO_2 conditions

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Ocean acidification (OA) is the result of increasing amounts of CO_2 entering the marine environment and is documented as having harmful impacts to a variety of marine organisms. When considering the future risk of hydrocarbon pollution, which is generally detrimental to marine life as well, we need to consider how OA induced changes to microbial communities will compound this since hydrocarbon degradation is influenced by community-level microbial response. This study aims to evaluate the effects of increased atmospheric CO_2 conditions and oil enrichment on *Emiliania huxleyi* and associated bacteria. *E. huxleyi* is an oligotrophic coccolithophore that is ubiquitously found in the ocean and harbours hydrocarbon-degrading bacteria that are associated with its phycosphere. *E. huxleyi* were cultured in ambient and elevated atmospheric CO_2 conditions for 5 days to establish exponential phase, before introducing 1% (v/v) of crude oil into the cultures to simulate oil spill conditions at sea. It was found that elevated CO_2 conditions do not significantly affect the growth rate of *E. huxleyi*, but exposure to crude oil detrimentally affected its growth rate compared to that under ambient CO_2 conditions. Elevated atmospheric CO_2 positively affect microbially influenced emulsification of hydrocarbon. Current work is being performed to examine the bacterial community response to crude oil under ambient or elevated CO_2 conditions, as well as biodegradation of the oil.

Cell biology of pathogen entry into host cells

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Fourth extracellular loop of meningococcal PorA causes altered expression of G1 cell cycle regulators in human brain microvascular endothelial cells

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Neisseria meningitidis (meningococcus) is one of the major meningitis-causing bacteria and is known for its ability to breach the blood-brain barrier (BBB). Meningococcus binds to the Laminin receptor (LAMR) on the surface of the endothelium, which is part of the BBB. The meningococcal surface proteins PorA and PilQ were previously identified as the bacterial ligands responsible for binding and, subsequently the LAMR-binding moiety of PorA was localised to its fourth extracellular loop (PorA-Loop4). Using a circularized peptide corresponding to PorA-Loop 4 derived from *N. meningitidis* MC58, the interaction of PorA-LAMR induces a specific cellular response in human brain microvascular endothelial cells (HBMEC) including G1 cell cycle arrest. Here, we investigated whether PorA-Loop4 treatment affects the PI3K/Akt pathway to cause the G1 arrest in HBMECs, especially via the expression of cyclinD1/CDK4, which control the transition from G1 to S phase. Immunoblotting and densitometric analysis confirmed that CDK4 expression was significantly increased in cells treated with PorA-Loop4 for 24 h compared to untreated controls. Moreover, CDK4 expression in the PorA-Loop4 treated cells was significantly increased compared to PI3K/Akt inhibitor (ZSTK474) treated cells. These data suggest that PorA loop4 affects G1 arrest through the P13K/Akt pathway via CDK4.

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Functional characterisation of (p)ppGpp synthetases: enzymesrequired for bacterial stress adaptation and survival

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Staphylococcus aureus is a Gram positive bacterium that can colonise humans asymptomatically but also invade as a pathogen. In the host, bacteria encounter nutrient stresses which trigger the activation of the stringent response. This response is a conserved mechanism by which bacteria adapt to environmental changes such as amino acid starvation. It is mediated by two nucleotides, ppGpp and pppGpp, collectively known as (p)ppGpp. These small alarmones have many binding targets ultimately causing the cells to enter a slow growing state. In S. aureus (p)ppGpp is synthesised by three members of the RSH superfamily: RSH, RelP and RelQ. RSH is a bifunctional enzyme with both synthetase and hydrolase activities and a C-terminal regulatory region. RelP and RelQ are both small monofunctional synthetases with no regulatory domain. The aim of this project is to research how these synthetases are regulated transcriptionally, translationally and post-translationally. In order to identify environmental signals that trigger the transcription of the synthetases, *lacZ* reporter fusions have been constructed. These strains have been used to quantify the altered transcriptional profiles of the synthetases under a number of different conditions that are known to induce the stringent response in Gram negative bacteria. This method will now be used to investigate the effect of novel stress conditions. Altogether, this work aims to provide insights into the synthesis of (p)ppGpp by these enzymes and thus contribute to the mechanistic understanding of the pathogenesis of *S. aureus*.

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Cell-cell communication between Plasmodium and host immune via exosomes

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Malaria, kills up to a million people each year, is caused by the protozoa of the genus Plasmodium falciparum (Pf). These vector-born parasites cycle between mosquitoes and humans and, in both contexts, are faced with an unstable and hostile environment. To ensure survival and transmission, the malaria parasite must infect and survive in the human host and differentiate into sexual forms that are competent for transmission to mosquitoes. We found, for the first time that Pf-infected red blood cells (iRBCs) directly exchange cargo between them using nanovesicles (exosomes). These tiny vesicles are capable of delivering protected genes to target cells.
Cell-cell communication is a critically important mechanism for information exchange that promotes cell survival. How Pf parasites sense their host environment and coordinate their actions, remain one of the greatest mysteries in malaria. Moreover, our understanding in the mechanism regulate human immune response to malaria infection is poor. Here, we found that malaria-derived exosomes carry remarkable cargo providing a secure and efficient mode for signal delivery. We developed an exosome tracking assay and could measure Pf exosome uptake by different cell types. Moreover, although early life-stages of Pf-iRBC are considered immunologically inert, our initial observations show that ring-stage derived exosomes are immunogenic. We show that exosomes can specifically activate and induce pro-inflammatory responses, resulting in interferon type I response. This is a new area of malaria research which may shed a light on the ability of malaria parasite to manipulate their host response.

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Poly (lactide-co-glycolic) acid based cholesterol-containing microparticles to prevent Streptococcus pneumoniae pneumolysin-mediated toxicity in vitro.

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Abstract
An interplay of bacterial and host factors works together to determine the clinical outcomes of pneumococcal infections. Pneumolysin, a toxin of Streptococcus pneumoniae plays a vital role in the pathogenesis of pneumococcal infections by facilitating bacteria invasion and colonization. Hence, toxin neutralisation alongside antibiotic therapy is an important clinical therapeutic strategy to be harnessed. In this study, we have used engineered Poly (lactide co-glycolic) acid (PLGA) based cholesterol-containing microparticles to protect cells from pneumolysin-induced membrane damage. The primary hepatocyte cell line HepG2 cells were treated with different concentrations of pneumolysin in the presence and absence of cholesterol-containing PLGA microparticles and cytoplasmic LDH release due to membrane damage in the presence and absence of the microparticles was assessed. We have also assessed uptake of the microparticles and its effect on cellular cholesterol level and membrane damage. Here, we have found pneumolysin forms pores in the membrane of HepG2 cells leading to the loss of LDH. Results have also shown the ability of cholesterol present in the PLGA-based microparticle to interfere with the pneumolysin-mediated membrane damage, evident by a reduction in LDH release. However, uptake of the microparticles affected the cellular cholesterol pathway evident by cellular cholesterol depletion, an activation of caspase-1 and an upregulation of lipogenic genes, through an unknown signalling pathway. Hence, PLGA-based cholesterol microparticle, a promising candidate for the development of an adjunct therapy to improve management of pneumococcal infections, requires for studies to ascertain its mode of action.

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Proteomic profile of human brain endothelial cells in response of *C. sakazkii*

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It is becoming difficult to ignore the pathogenicity of *C.sakazakii*, which is associated with severe and often fatal cases of infant meningitis and necrotizing enterocolitis. Formerly, the cytopathogenic interaction of *C.sakazakii* with host cells resulting in cytotoxicity and stimulation of an inflammatory immune response. Comparative proteomic analysis appears ideal for the examination of global changes and has widely been used to enhance the knowledge of differentially expressed functional and regulatory proteins. There have been limited studies on the molecular understanding of *C. sakazakii* meningitis in human brain. Therefore, the objective of this study was to identify and quantify expressed proteins from HBMEC in response to *C.sakazakii* meningitis isolate, particularly with regard to association of death cases. Generally, over 2500 proteins were quantitated in response to the *C.sakazakii* strain 767 of four biological replicates for each infection condition (60, 90 and 120 min). All the proteins that were predicted to be responsible for cell protection, nucleoside triphosphates synthesis and gatekeeping movement showed downregulated expression after exposure to meningitic isolate. In contrast, the proteins that upregulated over the exposure times were predicted to play a role in peptidase activity, regulation during differentiation, promotion of autophagic flux and inflammation. The increase in these proteins could be a reflection of brain cell- and astrocyte mediated enhanced cytokine production, and thus, enhanced inflammatory processing. This study assumed that many cell death processes would be initiated in response to the *C.sakazakii* meningitic strain, but these were still not enough to kill the brain cells.

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In vitro* cell culturing of *Cryptosporidium parvum

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Cryptosporidium parvum is a member of the phylum apicomplexa, is one of the causative agents of cryptosporidiosis and has high mortality incidence in malnourished children and AIDS sufferers. *C. parvum* is transmissible to a wide range of mammalian host organisms, including humans and livestock. Typically, the parasite colonises the gut, but often the hepatobiliary system and the lungs are also infected. *C. parvum* has a complex life-cycle with parasite transmission through environmentally hardy oocysts, however the relation between *Cryptosporidium* and its host is poorly understood. To develop effective treatments against cryptosporidiosis more insight into host-parasite interactions is required. There is therefore a need for a reproducible culturing system which can support the full life cycle of the parasite. This project aims to optimise existing host-parasite platforms used in research laboratories and develop novel 3D culturing methods. Model gut cells (HCT-8, Colo-680N) are grown in standard cell culture format (T-flasks) or in purpose-designed 3D systems to propagate infection. Besides the current HCT-8 intestinal cells, we are testing a panel of selected cancer cell lines for their ability to support parasite propagation. Preliminary data from investigations in neuroblastoma, breast and lung cancer cell lines, along with various GI tract and hepatobiliary cell lines, will be presented. Taken together, these approaches will go towards a low cost continuous culture system of *C. parvum* with high yields of oocysts. This will improve efforts to study the cell biology of this neglected tropical disease, leading to new and effective treatments.

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Unravelling host cellular functions employed by MRSA during intracellular survival.

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As a facultative intracellular pathogen, Methicillin-resistant Staphylococcus aureus (MRSA) is able to invade and proliferate within mammalian cells, including professional and non-professional phagocytes. This intracellular survival may be used by the pathogen to evade host immune responses as well as exposure to antibiotics. While most studies have focused on the virulence factors of MRSA, host molecular factors exploited by the pathogen during intracellular survival remain mostly unknown. Our research aims to identify novel host-directed therapies against MRSA by studying the host molecular and metabolic factors exploited by the pathogen during infection.

Employing unbiased genome-wide screens, subsequent bioinformatics and in silico analysis, we have identified several host-cell genes that could be potentially involved in S. aureus pathogenesis. Furthermore, our results show that silencing certain genes in HeLa cells increases host cell viability after MRSA infection whereas bacterial survival is significantly reduced. This suggests that S. aureus is able to modulate different molecular pathways of the host cell for its intracellular survival.

On the other hand, metabolomics approaches (Gas Chromatography Mass Spectrometry - GCMS, in combination with stable isotope labelling) indicate that the Tricarboxylic (TCA) cycle and lower glycolysis pathway are affected by the presence of MRSA within the cell. In our conference contribution, we will discuss these findings, which provide a better understanding of the MRSA-host cell interactions and may lead to novel treatments against this versatile pathogen.

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Investigating the susceptibility of dendritic cell subsets to infection with European porcine reproductive and respiratory syndrome viruses spanning the virulence spectrum

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant swine diseases worldwide. Current attenuated and inactivated vaccines are inadequate and the emergence of highly pathogenic PRRS virus (PRRSV) variants poses a threat to food security. An improved understanding of the viral-host immune interface is critical to support the development of improved vaccines. Experimental infection with highly pathogenic PRRSV-1 subtype 3 strains is associated with enhanced immune responses, which have been attributed to the more effective clearance of these strains compared to typical PRRSV-1. Ex vivo data has also shown that this increased virulence correlates with a broader cell tropism of myeloid lineage immune cells. We are currently investigating the causation of this correlation by assessing whether PRRSV-1, of varying pathogenicity, can infect dendritic cells (DC), which are not canonically a target cell. A two-step magnetic-based cell sorting protocol and a multiparameter flow cytometric staining panel were optimised to enrich and identify the two major conventional (cDC1 and cDC2) and plasmacytoid (pDC) dendritic cell populations from porcine blood. I will present the results from ongoing experiments assessing the susceptibility and responses of individual DC subpopulations to infection with a highly pathogenic PRRSV-1 subtype 3 strain (SU1-Bel), a low pathogenicity PRRSV-1 subtype 1 strain (215-06) and a MARC-145 cell attenuated PRRSV-1 subtype 1 strain (Olot/91).

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Development of animal infection models to test the potential of pyocins as protein antibiotics

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In the past few years there has been a worldwide rise of bacterial antibiotic resistance. New therapeutic agents are now urgently needed to treat multi-resistant and chronic infections. Amongst those threats, *Pseudomonas aeruginosa* is a Gram-negative pathogen frequently resistant to conventional antibiotics and responsible for severe hospital-acquired blood and lung infections. A promising strategy is the development of bacteriocins, such as the *P. aeruginosa* expressed pyocins, as protein antibiotics. Previous work on pyocins (S2, AP41, S5 and L1) has demonstrated their activity against a pathogenic strain of *P. aeruginosa* in a murine (C57Bl/6) model of acute lung infection on post-infection administration. However, the lack of understanding of the entry of pyocins into the cell means that it is currently difficult to circumvent strains specificity and the emergence of resistance, making it difficult to develop these proteins as antibiotics. Our current goal is to further our understanding of pyocins mechanism and demonstrate the efficacy of treatments with pyocins in blood or lung murine models of infections.

Circadian Rhythms

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Investigating the circadian clock of *Verticillium dahliae* and its influence on pathogenicity

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Many organisms have evolved to adapt themselves to the rhythmicity of their environment. A broad knowledge of the molecular mechanism of circadian clocks has been gained through the fungal model *Neurospora crassa*. Nevertheless, little is known about circadian clocks in other fungi. *N. crassa* is related to many important plant pathogens including the vascular wilt *Verticillium dahliae.*

Having identified homologues of the *N. crassa* clock oscillator proteins in *V. dahliae,* we found high conservation in clock protein domains, which suggested the existence of a functional clock. However, no evidence for an entrainable, free-running rhythm was observed in the daily formation of conidia and microsclerotia. Deleting core clock genes, such as *frq,* does not have any obvious effect on fungal morphology. Furthermore, temporal gene expression profiling showed a lack of rhythmic *frq* expression. The deletion of the TF/photoreceptor WC-1 prevents ring production. Thus, WC-1 is involved in the production of the daily developmental rhythm by mediating transcriptional responses to light. We performed an orthology analysis with 29 Sordariomycetes species and investigated the presence of a *N. crassa* Clock Box in the *frq* promoter of *V. dahliae*. The C-Box (5'CGATCCGCT3') is essential in regulating rhythmic expression of *frq* in *N. crassa*. A similar motif (5'CGATCCCCT 3') was found in *Verticillium spp*, and is present in other related species of the Sordariales.

In conclusion*, V. dahliae* presents all the necessary genetic loci for a functional clock, but there is no evidence of rhythmicity in either morphological traits or in gene expression.

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Investigation of Candida diversity in an infertile cohort using next generation genetic analysis

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Vaginal candidiasis affects 75% of all women at least once during their life with the main causative species, Candida albicans, also implicated in infertility. Recent in vitro studies show that Candida albicans influences spermatozoa motility suggesting a role for C albicans in male infertility. However, few studies have investigated Candida species diversity and its impact on infertility.

Candida prevalence and diversity from the oral and female/male reproductive tracts of 16 couples (32 individuals) were cultured using a selective media. Eighty-three yeast isolates were characterised phenotypically. Amplification and pyrosequencing of the internal transcribed spacer 2 (ITS2) was used to genetically identify Candida species. Multilocus sequence typing (MLST) analysis was used to assign sequence type among the culturable C. albicans isolates.

Candida species were present in the oral samples of 93% of the women, however only 43% of men were colonised. C. albicans accounted for 73% and 57 % in women's and men's oral samples, respectively. A high diversity of oral yeast (7 species) was identified in the present cohort. Candida colonisation in semen was lower 6%, in comparison with 37% in vaginal samples. C. albicans (66.6%) was the predominant species in the vaginal samples,. Only one couple shared Candida albicans according to ITS2 profile across all body sites. However, MLST analysis identified a greater intra-species diversity in oral and reproductive tract isolates. These findings suggest a higher diversity of Candida species present in oral but not the reproductive tract isolates. Ongoing work will link this to fertility status of the couples.

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"Isolation, Speciation and Antifungal Susceptibility Testing Of Candida Isolates from Various Clinical Specimens at a Tertiary Care Hospital, Nepal"

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Background: Candida species are responsible for various clinical infections ranging from mucocutaneous infection to life threating invasive diseases. CHROM agar media can be reliably used for speciation of Candida isolates which helps to rapid identification of Candida species. The objective of the present study was to determine different species of Candidaand to determine antifungal susceptibility pattern.

Methods: A total of 100 consecutive Candida isolates from various clinical samples were studied. Growths on Sabouraud's Dextrose Agar were evaluated for colony appearance, macroscopic examination, gram staining, germ tube test and urea hydrolysis test. They were further processed for Candida speciation on CHROM agar. Antifungal susceptibility testing was performed and interpreted for all the isolates using disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) M44-A document.
Results: Out of 100 Candida isolates, Candida albicans (56%) was the most common species. Among the non-albicans candida (NAC), Candida tropicalis (20%) was the commonest isolate. Overall susceptibility pattern of Candida species to clotrimazole found to be more sensitive (82%) whereas ketoconazole was found to be more resistance (86%).
Conclusions: Candida albicans was the predominant species responsible for various candidal infections. CHROM agar is a simple, rapid & inexpensive method for identification of Candida species and is suitable for clinical laboratory with limited resources.

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Category: Poster presentation

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A giant impact: elucidating the role of *Candida albicans* cellular gigantism on host-pathogen interactions

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Candida albicans is a major human fungal pathogen, responsible for superficial, as well as life-threatening disseminated infections. Polymorphism in *C. albicans* is an established virulence attribute and fungal cells must acquire essential nutrients from the host in order to proliferate. However, the host immune system has evolved complex mechanisms to actively withhold essential nutrients like zinc in a process known as nutritional immunity. Therefore, in the battle of pathogenicity versus nutritional immunity, the fungus must have developed strategies to survive and thrive within the host under the restrictive conditions of nutritional immunity. We have observed that *C. albicans* transforms to a giant 'Goliath' yeast morphology upon zinc starvation. This response is also seen in multiple *C. albicans* clinical isolates and in the related species, *Candida dubliniensis* and *Candida tropicalis*. Investigations into the physiological properties of Goliath cells has revealed cell wall alterations , increased lipid droplets, enlarged vacuoles, delayed hyphae formation and increased adhesion to abiotic surfaces. With such prominent differences in virulence-associated properties, we are now exploring the interactions of Goliath cells with human epithelia and immune cells.

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Exploring adaptations to iron starvation in Aspergillus fumigatus

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During infection, invading microorganism and host enter a struggle for iron on which the outcome of infection is decided. Aspergillus fumigatus is the predominant causative agent of life-threatening Invasive Aspergillosis and possesses sophisticated adaptations to iron starvation. Importantly, the production of iron-chelating siderophores in vivo is essential to virulence. Characterisation of the iron-starved proteome of A. fumigatus is, therefore, warranted to understand the role of iron at this host: pathogen interface and to dissect the basis of siderophores as therapeutic targets

A. fumigatus was grown in iron-replete and -deplete conditions, and secreted, microsomal, and mycelial protein fractions were extracted and analysed by Label-Free Quantitative proteomics. Microsomal (n = 96), mycelial (n = 789), and secreted (n = 182) proteins with increased abundance ($p < 0.05$) under iron limitation were identified. Additionally, microsomal (n = 135), mycelial (n = 512), and secreted (n = 238) proteins with decreased abundance were identified. Functional categories significantly represented ($p < 0.05$) in the iron-starved proteome included cell wall organisation, secondary metabolite production, cellular respiration and transport, including putative siderophore transporters. Siderophore, fusarinine C (FSC) was RP-HPLC purified and fluorescently derivatized. This permitted visualisation of the uptake and subsequent fate of the iron: siderophore complex, and provides a method to evaluate the therapeutic strategies that impede siderophore uptake.

This work has shed light on the networks of proteins affected by iron limitation in A. fumigatus. Specifically, the cellular localisation and putative proteins mediating the fate of siderophores in A. fumigatus have been further unveiled.

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Role of the Cst20 kinase in the regulation of hyphal steering in *C. albicans*

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Candida albicans is a commensal fungus that can cause fatal infections in patients undergoing immune-suppressive therapy. An essential pathogenicity trait of this fungus is its ability to form invasive hyphal filaments that penetrate host tissue and establish deep-seated infection. Hyphal ingress into host tissue requires specific growth characteristics, including the ability to steer and maintain a penetrative growth trajectory. We have identified 3 defective 'steering' phenotypes in *Candida albicans* hyphae, which are produced by deletion or mutation of specific proteins. Deletion or hyper-activation of the small GTPase Rsr1 produces an erratic growth trajectory, loss of hyphal steering responses and reduced tissue penetration. Deletion of the Paxillin-like gene, *PXL1*, or its putative kinase, *PTK2*, produces wavy hyphae accompanied by partial loss of contact-dependent (thigmotropic) steering responses. Truncation of the kinase domain, but not deletion, of Cst20 or hyper-activation of Rho1 results in straight hyphae that are unresponsive to all external cues tested. Intriguingly, although the wavy and straight hyphal phenotypes are morphological opposites, other phenotypic defects, including impaired cytokinesis, are similar in both *pxl1*Δ and Cst20$^{\Delta 1\text{-}549}$ mutants, suggesting that they act in at least one shared pathway. The Cst20$^{\Delta 1\text{-}549}$ mutant was found to hyper-activate human macrophages and was subsequently taken up faster than control cells, suggesting heightened shedding or secretion of cell components by this mutant. We are investigating the hypothesis that the N-terminal of Cst20$^{\Delta 1\text{-}549}$ sequesters a small GTPase that is required for constant cycling to allow hyphae to adjust their direction of cell growth in response to environmental cues.

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Pneumocystis jirovecii induced chronic interstitial lung disease in Waldenström's macroglobulinemia that was followed by lung adenocarcinoma

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Introduction: Infections with the fungus Pneumocystis jirovecii can result in asymptomatic colonisation in otherwise healthy patients, but induce life threatening clinical symptoms known as Pneumocystis pneumonia (PcP). However, there appears to be a "grey area" between colonisation and severe pneumonia that remains underestimated so far. Here we describe a case with chronic interstitial lung disease and chronic cough that was attributed to Pneumocystis jirovecii.

Case Presentation: The patient was a 67 year old male who presented with non-acute Morbus Waldenström, dyspnoea on exertion and chronic cough. Initially, interstitial lung disease was suggested to be related to hypersensitivity pneumonitis, but the patient's condition worsened when receiving oral corticosteroids. Subsequently, chronic Pneumocystis infection was diagnosed that, however, did not progress to pneumonia. Of note, interstitial lung disease substantially improved following anti-fungal treatment. During the further course, adenocarcinoma of the lung was detected that was successfully treated by surgery.

Conclusion: The patient's history of chronic cough, although very likely being fostered by the underlying Waldenström's macroglobulinemia and interstitial lung disease, was most likely caused by Pneumocystis jirovecii infection. Thereby, the fungus did not induce pneumonia despite the immunomodulation caused by the Waldenström's syndrome, but was associated with interstitial lung disease that responded to specific treatment This gives raise to the hypothesis that P. jirovecii infections do not necessarily induce life threatening pneumonia. Consequently, serial testing is required in eligible patients with positive PCR results in order to discriminate between colonisation, "grey zone" infection, and beginning pneumonia.

Epigenetic and Non Coding RNAs in Eukaryotes

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Functional profiling and genetic interaction map of non-coding RNAs in yeast

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Background: A protein deletion collection in *Saccharomyces cerevisiae* has been proven to be useful for functional genomics analyses, however little is known about the molecular function of non-coding RNAs (ncRNAs). We have created a collection of ~450 ncRNA knock-out (KO) mutants in *MATa* that were used for analysis in this study.

Aims: To create a genetic interaction map of ncRNAs in *S. cerevisiae* by (*i*) fitness analysis of double KO mutants generated using Synthetic Genetic Array (SGA) and (*ii*) screening the double KO library in stress conditions.

Methods: Double KO mutants were generated using SGA by crossing a query strain (carrying single gene deletion) with the ncRNA KO collection. Colony sizes of the single and double KO mutants were recorded. Generated negative and positive scores that represented gain or loss of fitness were used to create scatter-plots and genetic network. Fitness analysis is being performed in various conditions.

Results: To date, ~100 query strains have been generated carrying deletions in snRNAs, SUTs, CUTs and tRNAs. Thirty have been used in SGA to generate double mutations. Several gene interactions have been discovered showing either loss or gain in fitness, such as for example ΔSUT193-ΔSUT055 or ΔSNR13-ΔSUT347. Genetic networks for discovered interactions will be presented as well as fitness results of double KOs.

Conclusions: The SGA analysis on a small number of query strains (30) showed a number of positive and negative genetic interactions, with a few being lethal. Phenotypic analysis of double mutants will help us determine the function of ncRNAs.

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Gaining insight in the physiological role of small RNAs expressed in Burkholderia cenocepacia

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BACKGROUND: Burkholderia cenocepacia is an opportunistic respiratory pathogen. To survive inside the host, bacteria (including B. cenocepacia) need a coordinated regulation of their gene-expression. Small non-coding RNAs regulate gene expression on the post-transcriptional level and are still uncharacterised in B. cenocepacia.
METHODS: We identified 60 putative sRNAs expressed in B. cenocepacia strain J2315, using differential RNA sequencing. qPCR and northern blotting were used to confirm the expression and the size of 11 sRNAs. To investigate the physiological role of one of these, sRNA nc35, a deletion mutant, Δnc35, was constructed. Various characteristics, including biofilm formation, metabolic activity and susceptibility to various stresses were determined. Finally, RNA sequencing was performed to analyse gene expression.
RESULTS: Different phenotypes are observed comparing wild type strain to Δnc35 mutant. When grown as a biofilm in 96-well plates, mutant cells formed larger aggregates than WT cells. In planktonic cultures, the mutant grows faster and to a higher OD in various media and it shows a higher metabolic activity. In MIC tests, the mutant is more susceptible to ceftazidime and meropenem. RNA sequencing reveals an upregulation of tryptophan and phenylacetic acid degradation pathways in planktonic cultures, whereas in biofilms, genes involved in stress response are upregulated.
CONCLUSION: We identified and confirmed the expression of 11 small RNAs in B. cenocepacia J2315. Deletion of sRNA nc35 resulted in a mutant with an altered phenotype and a changed gene expression. The most obvious trait of the Δnc35 mutant was a faster growth rate and metabolism, suggesting the sRNA can suppress growth.

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The Role of Long Non-coding RNAs in Human Cytomegalovirus infection

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Recent advances in genomics and transcriptome studies have revealed that a large proportion of the mammalian genome does not code for any protein. While short non coding RNAs like miRNA have been well studied, relatively less is known about long non-coding RNA (lncRNA). LncRNA are defined as non-protein coding RNA that are longer than 200 nucleotides in length. Growing evidence of their involvement in gene regulation and key cellular processes, make them an appealing RNA species to study. Little is known about the role of lncRNAs in the context of virus infection. Here we employ two distinct strategies to study their role in infection with human cytomegalovirus (HCMV), a medically important herpesvirus.

In the first approach we use a high-throughput siRNA screen against 2231 lncRNAs to determine the effect of knockdown on HCMV. Using a siRNA library against 2231 human lncRNA genes, we employ a two-part screen that allows us to identify lncRNA involved not just in virus entry and replication but also in virus assembly and egress. Our data shows 6 siRNAs that reduce primary infection and 4 that reduce virus production. In a different approach we examine expression data from RNASeq performed on cells infected with HCMV and look at lncRNAs that are differentially expressed during infection. We observed several lncRNAs that were differentially expressed and have chosen 10 for further validation. Taken together these studies aim to elucidate the role of host lncRNAs in HCMV viral replication.

Epigenetic and Non Coding RNAs in Eukaryotes

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Development of Small Interfering RNAs to Treat Fungal Disease

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Aspergillus fumigatus is a ubiquitous saprophytic fungus that causes life-threatening disease in immunocompromised individuals. The increasing resistance to current antifungals makes the development of new antifungal drugs an important goal. Our research focuses on inhibiting fungal growth by activating the RNA interference (RNAi) pathway. Activation of the RNAi pathway by dsRNA results in degradation of target mRNA. Therefore this pathway can potentially be exploited to target essential fungal genes leading to their down-regulation, resulting in growth inhibition or death. Major barriers to siRNA entry into the cell are the fungal cell wall and cell membrane. If these obstacles can be overcome a new class of antifungals based on siRNA could be developed.

We assessed the uptake of siRNAs and siRNAs attached to cell-penetrating peptides by A. fumigatus and the model filamentous fungus Neurospora crassa. In a variety of conditions, confocal microscopy reveals siRNAs accumulate in the cell wall of A. fumigatus and cell wall, septa and hyphal tips of N. crassa. To-date, unmodified siRNAs even as small RNAs with chemical modifications (PNA, 2'- fluoro2- deoxy, 2'-O-methyl and/or phosphorothioate linkages) have had no significant impact on growth or target mRNA levels. We are presently testing the efficacy of pooled siRNAs complementary to a variety of short sequences in the target mRNA.

Epigenetic and Non Coding RNAs in Eukaryotes

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The Grc RNAs are a novel family of antisense regulatory RNAs that regulate the replication of the Gifsy bacteriophages of Salmonella Typhimurium and the viral lysis-lysogeny decision.

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The cis-encoded antisense RNAs (asRNAs) of bacteriophages play a key role in the control of diverse biological functions. In the temperate phage λ of Escherichia coli, the OOP asRNA is encoded antisense to the cII gene, and overlaps with the 3' end of the cII mRNA. OOP binds to the CII mRNA, leading to the degradation of the transcript and a reduced level of the CII protein, a pivotal regulator of the λ lysis-lysogeny decision.

A recent RNA-seq analysis identified OOP-like asRNAs encoded within the Gifsy lambdoid prophages of the human enteropathogenic bacterium Salmonella Typhimurium. We identified three different versions of the asRNAs encoded within the Gifsy-1, Gifsy-2 and Gifsy-3 prophages, namely GrcX, Y and Z, respectively (Gifsy replication control). Like OOP, all the Grc RNAs are encoded tail-to-tail to cII and head-to-head to the O gene, which itself encodes the Gifsy O replication protein. Unlike OOP, the antisense transcription of the Grcz asRNAs does not overlap with the 3' coding region of the cII mRNA, suggesting a novel regulatory function in the control of the Gifsy phage biological functions.

The Grc-based regulation of Gifsy phage biology is completely different to the OOP-driven regulation observed in the archetypical phage λ. We will explain the impact of the Grc asRNAs upon the replication of Gifsy phages, and show that these new asRNAs influence the viral lysis-lysogeny decision.

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A novel phase-variable epigenetic regulatory mechanism in Listeria monocytogenes that affects bacterial cell division

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Recently, a phase variable Type I Restriction Modification (RM) system that epigenetically regulates virulence phenotypes has been identified in Streptococcus pneumoniae. The system contains single hsdR (restriction) and hsdM (methylation) genes and multiple hsdS (specificity) genes, which by recombination alter the DNA target of the restriction-methylation complex. This study investigates the presence and function of a similar novel epigenetic regulatory mechanism in the food-borne pathogen L. monocytogenes, which causes human listeriosis.
Bioinformatic analyses have identified three families of Type I RM systems amongst the L. monocytogenes genomes available in GenBank. Only one of these is able to recombine under different environmental conditions and generate different methylation patterns (thus indicated as phase-variable). Interestingly, genetic screening of a panel of 100 human invasive disease and 150 food isolates revealed a statistically significant association of the Type I RM phase-variable system to human disease. Moreover, sequence analysis of the phase-variable system showed evidence of distinct variants amongst the target recognition domains of the hsdS alleles, which were conserved and associated to specific clonal complexes. A PCR-based protocol was designed and validated to quantify the expressed hsdS alleles and monitor population structure changes under selected conditions. Growth of bacteria at varying temperatures (6-37°C) revealed that hsdS alleles recombination was maximal at 22-24°C, while colonies remained largely mono-allelic at 37°C. Single hsdS allele-rich populations were then isolated and are being used to identify DNA methylation targets and examine allele-specific phenotypes. Preliminary results indicate that this epigenetic regulatory mechanism might affect bacterial morphology and cell division.

Synthetic and Systems Biology Approaches to Microbiology

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RCGC: Remote Control of Genetic Circuits

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In the future, biotechnological processes may be absolved of chemical induction methods. Instead genes and biosynthetic processes could be controlled remotely by radio waves. To this end, we are developing an array of bacterially produced "aerials" and responsive elements for use in the remote control of gene expression.

Agrobacterium tumefaciens uses a temperature sensitive two-component system called VirAG that acts as the master regulator of its virulence plasmid. Using synthetic biology methods, a magnetic nanoparticle aerial could be attached and excited using radiofrequency electromagnetic waves, generating heat which could turn this sensor off. There are a wealth of bacterial ferritins and other proteins that produce and store magnetic iron. By fusing the temperature sensitive VirA with one of these a genetic remote control is generated.

E. coli is also capable of producing palladium nanoparticles using its hydrogenases. Bringing these nanoparticles in to proximity of VirA and exciting them using a resonance frequency at their diameter, heat may also be emitted again affecting its activity as a sensor.

Constructing these systems in *E. coli* opens up an array of existing useful biochemical processes to be controlled downstream. These methods could be applied in large biotechnological processes to produce valuable and useful products, or to communicate with and control bacteria from great distances.

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Biocontainment of Bacteria for Vaccine Delivery

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Genetically modified organisms (GMOs) are confined physically in order to contain their spread in nature and to minimise chances of horizontal gene transfer. However, with the potential that GMOs hold as cheap, reliable and efficient micro-machines with the increase in applications for synthetic biology, their eventual release into the wider space is predictable.

A major potential application of GMOs is the delivery of vaccines to human and animal hosts, through the utilization of engineered microbes. Recombinant technology is promising for a number of reasons including their capacity to be less reactogenic, more potent, safer and genetically definable. Also, they have the potential to provide protection against multiple targets simultaneously, are economically affordable and can be eradicated with antibiotics as the need arises.

Mutant *Salmonella* expressing heterologous antigens have been shown to induce protection against a variety of pathogens. This project aims to design a *Salmonella* vehicle for vaccine delivery which self-destructs upon exit from the host. An orthogonal translation system has been constructed to eliminate antigen translatability, if horizontal gene transfer occurs. In addition, temperature-sensitive mRNA and inteins can be used as control switches for endonuclease and RNase expression, to facilitate internal cell destruction, thereby limiting risks of genetic release and horizontal gene transfer. Progress towards these aims will be described.

Annual Conference 2017
EICC, Edinburgh
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DEVELOPMENT OF A TRANSPOSON MUTAGENESIS SYSTEM FOR METHANE OXIDISING BACTERIA

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DEVELOPMENT OF A TRANSPOSON MUTAGENESIS SYSTEM FOR METHANE OXIDISING BACTERIA

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Methane oxidising bacteria are diverse and environmentally widespread microorganisms that can grow using methane as their sole source of carbon and energy. There is much interest in developing them for numerous biotechnological applications including production of fine chemicals and bioplastics, to produce biological products such as recombinant proteins from methane as the feedstock and for bioremediation of diverse organic and inorganic pollutants. This study aims to develop a system to allow study of the physiology of methanotrophs by construction of transposon libraries of inactivated genes. A conjugative plasmid containing the mariner transposon has been introduced into the model methane oxidising bacterium *Methylosinus trichosporium* OB3b. The progeny from the conjugation were kanamycin resistant (strongly suggesting they contain the plasmid) and resistant to nalidixic acid (confirming removal of the *Escherichia coli* donor strain). Subject to the construction of a suitably large transposon library, the system will be used to investigate the genetic basis of metal and metalloid bioremediation in methanotrophs.

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Metabolic Engineering of *Geobacillus* for production of important platform chemicals.

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The global use of fossil fuels as our major energy source has led to accumulation of greenhouse gases and concerns regarding climatic changes. One potential solution to overcome this issue, is use of microorganisms to utilise renewable resources or waste products to produce fuels and important platform chemicals.The work aims to engineer a thermophilic bacterium '*Geobacillus thermoglucosidasius*' to utilise lignocellulosic biomass, an abundant waste product, to produce biofuels and platform chemicals. This work also focuses on production of 3-Hydroxypropoinic acid (3HP), a promising bio-based platform chemical. Bio-based production of 3-HP could therefore decrease the dependence on fossil fuels and contribute towards a sustainable bio-based economy. The chemical 3-hydroxypropionic acid (3HP) is an important building block and is ranked among the top third of the 12 platform chemicals selected by the US Department of Energy. Recently, there has been great interest in producing 3HP at an industrial scale from renewable sources, instead of via traditional chemical synthesis. We are engineering the strain using synthetic biology approach for production of 3HP.

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Characterising interactions in human vaginal microbiomes to investigate potential metabolic links between medical syndromes.

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The human body is colonised by an immense number of microbial organisms inhabiting various tissues and body sites and although most microbiomes are beneficial for the host, environmental disturbances can lead to negative clinical consequences. Microenvironment disruption has been linked with various disorders in the vaginal tissue including Bacterial Vaginosis, HIV and Sexually Transmitted Infections. Microbiome studies have proven a useful tool in characterising microorganisms associated with health and disease in humans. Amplicon data can provide information on the relationship between bacterial community composition and ecosystem function. This study aims to identify correlations between members of the vaginal microbiomes from different individuals with genealogical disorders, to gain insight into the microbial interactions that affect community assembly. Although positive and negative correlations between bacterial taxa may give us some insight, it can be enhanced by exploring the metabolic properties of these taxa. A pipeline was designed to allow cultivation-free, bioinformatics analysis on existing amplicon data from vaginal microbiome studies. Qiime (Quantitative Insights Into Microbial Ecology) and other purpose-written Python scripts were designed to complete taxonomy assignment, diversity and clustering analysis, as well as to assess the statistical significance of the correlations from the interactions observed. Analysis thus far suggests strong correlations between some bacteria, which are also reinforced by the presence of metabolic links. The findings show the prospect for identifying links between microbiome and pathogenicity and thus gaining an understanding on vaginal microbiome composition and structure particularly in the gynaecological syndrome of bacterial vaginosis.

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A Hypertransformable *Clostridium pasteurianum* and Its Use for Genetic Engineered Improvement of Butanol Production

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Clostridium pasteurianum is a Gram-positive spore forming bacterium with the ability to produce the biofuels ethanol, *n*-butanol and the bioplastic precursor 1,3-propanediol (PDO) from the biodiesel by-product glycerol. In recent years application of synthetic biology to this organism for improved product yields attracted research interest and improved transformation efficiency would accelerate this development. Here we present a straightforward method to screen for sub-populations of this species that are electro-transformable with high efficiencies compared to wild type. We then show that only one SNP is responsible for the hypertransformable phenotype and present reasons for its involvement.

Further, the central energy metabolism of this mutant was genetically engineered. Glycerol is used reductively to produce PDO and oxidatively to produce acids and solvents. We deleted the first gene in the reductive branch (*dhaBCE*) to channel carbon towards solvents. Growth difficulties of this strain under bioprocess conditions accentuated the importance of this pathway for redox homeostasis during glycerol fermentation. We furthermore inactivated the main hydrogenase HydA, hitherto thought essential in clostridia, and increased ethanol and *n*-butanol titres. Finally, we knocked-out the redox response regulator Rex, responsible for regulation of intracellular NADH/NAD^+ levels, which increased alcohol levels as predicted by our *in silico* analysis of Rex target sites.

We believe the screening method for hypertransformable mutants to be applicable in a wider range of organisms and with the improvement of *n*-butanol production by inactivation of *hydA* and *rex* we place *C. pasteurianum* as an important chassis for the industrial production of this important chemical.

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High cell density fermentation of microorganisms for production of chemicals. Lois Henderson, Ingenza Ltd, Roslin BioCentre, Roslin, EH25 9PP, UK.

Lois Henderson
Ingenza Ltd, Edinburgh, UK

Ingenza is a synthetic biology/industrial biotechnology R&D company with a broad customer base across the chemicals, pharmaceuticals, food/feed and fuel industries. Our customers include (i) chemical companies looking to source capabilities in microbial/molecular biology and bioprocess development for the production of biobased chemicals and biofuels, (ii) therapeutics companies, looking to outsource the application of synthetic biology for natural product pathway and/or protein engineering/optimization and (ii) other SMEs/academics looking to transition early stage research through proof of concept and on to spinout/startup in human therapeutics. The Fermentation Department is central to all of Ingenza's business areas and plays an integral role within the company. This talk is to illustrate the key role of fermentation within synthetic and systems biology approaches for industrial applications. The focus will be on the development and optimisation of a high cell density fermentation process for the production of chemicals. I hope my talk will allow the audience to better appreciate how fermentation processes compliment synthetic biology.

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All bacteria in a population are equal, but some bacteria are more equal than others

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Persister cells are responsible for chronic infections relapse and have been proposed to play a role in antibiotic resistance. They exploit phenotypical differences to tolerate antibiotic insults, often involving a reduction of the growth and metabolic rate. The energetics accompanying this phenotypical switch remain largely unexplored. Understanding the energy fluxes involved in persistence may disclose novel drug targets and parameters useful for diagnostics.
In the present study, utilizing molecular biology techniques and fluorescence microscopy, we develop and optimize assays to measure E. coli's energy fluxes in real time and on the individual cell level, with the aim of applying them to study persister cells.
Building on the work by Imamura et al., we have successfully optimized the expression of the QUEEN ATP concentration sensor, which we can now use for dynamic measurements. To asses membrane voltage, we have developed a protocol allowing the use of the established Nernstian dye TMRM also in growth media. In addition, we have characterized the newly proposed membrane voltage dye, Thioflavin T.
Applying these methods allows us to draw the energetic identikit of E. coli single cells and assay their physiology at different growth stages and under different stresses, such as antibiotics. In such a way we are able to observe energetics of persister cells during their formation.

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Extending Product Streams: Converting Clostridium acetobutylicum from an ABE to IBE Producer

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Background
C. acetobutylicum is an attractive industrial chassis for the production of biofuels and in the ABE fermentation produces Acetone, Butanol and Ethanol (3:6:1). The aim of this work is to extend the product streams of this organism converting ABE to IBE (Isopropanol, Butanol, Ethanol) using genetic tools developed in the Clostridia Research Group, Nottingham.

Method
A triple auxotrophic mutant which represents alternative sites that allow the introduction of genes encoding pivotal pathway enzymes and orthogonal expression system using appropriate Allele Coupled Exchange (ACE) vectors is being created. With the introduction of isopropanol operon at a locus, acetone is converted to isopropanol in one step by a secondary alcohol dehydrogenase.

Results
Suicide plasmids with a functional pyrE gene was used to knock out argH (argininosuccinatelyase) and pheA (prephenate dehydratase) genes from the C. acetobutylicum ΔpyrE mutant. Double mutants were made and appropriately complemented. Data obtained to determine the locus specific effects on gene expression showed highest expression at the pyrE locus followed by the pheA and least at the argH, likely based on the relative proximity of the locus from the chromosomal origin of replication.

Conclusion
The creation of loci at which desired operons can be incorporated will allow the production of the more useful fuel additive, isopropanol from C. acetobutylicum.

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Towards carbon efficient utilisation of D-xylose for chemicals manufacturing

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Utilisation of glucose for bio-based manufacturing of chemicals is unsustainable because it is produced mainly from human foodstuffs. D-xylose provides a better alternative, since it is available from food and agricultural wastes. However, for chemicals production, there is frequently a need to form TCA cycle intermediates before elaboration to the final target, and assimilation of D-xylose via the classical pentose phosphate pathway (PPP) is inefficient. The PPP channels D-xylose into glycolysis via D-glyceraldehyde-3-phosphate and, thence, to pyruvate. Subsequently, pyruvate is oxidized to acetyl-CoA, causing wasteful loss of carbon as CO_2. This is a serious problem for chemicals manufacturing where atom economy is crucial both for sustainability and economics.
An alternative route where D-xylose is converted to α-ketoglutarate without carbon loss exists, discovered in Pseudomanas sp.[1] and subsequently characterised in Caulobacter crescentus[2]. In this study, we optimised an expression system for this pathway in E. coli by using the C. crescentus genes xylXABCD, and Ingenza Ltd's propriety combinatorial DNA assembly method (inABLE®), testing different promoters and varying the order of the genes in the synthetic operon. As a result, although the maximum growth rate was only 40% of wild type strains using the classical PPP, for the first time, we have identified strains that could grow constitutively on D-xylose using the new pathway. These strains provide a platform for carbon efficient production of a plethora of chemicals from D-xylose, through the addition of new pathway modules.

[1] Weimberg 1961. J Biol Chem 236:629:635
[2] Stephens et al. 2007. J Bacteriol 189:2181:2185

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Cell to cell communication in Clostridium acetobutylicum involved in the regulation of early solvent formation.

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The strictly anaerobic bacterium Clostridium acetobutylicum is well known for its ability to convert sugars and starches into acids and solvents, most notably the potential biofuel butanol. However, the regulation of its fermentation metabolism, and in particular the shift from acid to solvent production, remains poorly understood. The aim of this study was to investigate whether cell-cell communication (quorum sensing) plays a role in controlling the timing of this shift or the extent of solvent formation. Analysis of the available C. acetobutylicum genome sequences revealed the presence of eight putative RNPP-type quorum sensing systems, each consisting of RNPP-type regulator gene followed by a small open reading frame encoding a putative signalling peptide precursor. Three independent regulator mutants were generated for each system in C. acetobutylicum ATCC 824 and screened for phenotypic changes. One of the RNPP-type regulator mutants, designated qsrB, was of particular interest as they showed increased solvent formation during early solventogenesis. Overexpression of qsrB considerably reduced solvent and endospore formation, suggesting that it is acting as a repressor. Addition of short synthetic peptides representing internal fragments of the presumed cognate peptide (QspB) counteracted QsrB-mediated repression and restored both solvent production and sporulation. Together, these findings support the hypothesis that QsrB and QspB form a functional quorum sensing system, which is involved in the regulation of early solvent formation and endospore formation.

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BIOREMEDIATION OF HEXAVALENT CHROMIUM BY METHANE OXIDISING BACTERIA

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BIOREMEDIATION OF HEXAVALENT CHROMIUM BY METHANE OXIDISING BACTERIA

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Methane-oxidising bacteria are widespread in the environment and have a number of promising activities for bioremediation of organic and inorganic pollutants. One of these activities is the ability of some methanotrophs to remediate chromium VI by reducing it to the less toxic and less bioavailable chromium III, although the enzyme(s) responsible for this reaction have not been identified. This study aims to identify and characterise the chromate (VI) reductase of Methylococcus capsulatus (Bath) and to isolate new chromium (VI)-bioremediating strains of methane-oxidising bacteria from the environment. Cells of Methylococcus capsulatus (Bath) were fractionated to separate them into cytoplasm, cell wall and cell membrane fractions. All fractions reduced chromium (VI) to chromium (III), and the reduction activity was greatest in the cytoplasm fraction. The reductase has been partially purified from the cytoplasm fraction by using a 4.7-mL Hiscreen Capto DEAE anion exchange column and it has been shown that its chromium VI-reducing activity is enhanced by adding the electron donor NADH. The reductase is now being further purified by using Blue Sepharose affinity chromatography. A sample of canal sediment from a location in northern England has been used to inoculate an enrichment culture growing at 45ºC on methane and a new chromium (VI)-reducing strain was isolated and shown to be another strain of Mc. capsulatus.

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Prospecting the rumen protozoa for novel cellulases, lipases and proteases using metagenomic techniques

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Protozoa, along with fungi, represent the rumen eukaryotome and account for up to 50% of microbial biomass. Nonetheless, they are often overlooked. It is inferred that rumen protozoa play a role in lipid, protein and fibre metabolism, however, few of these enzymes have been identified and characterised. Enzymes previously isolated from the rumen microbiome have shown great potential for application in industry (biodiesel manufacture, the food industry, waste management etc.) suggesting a wealth of untapped, novel activity in the rumen eukaryotes.

In order to capture the full functional capacity of the rumen protozoa, a metagenomic approach was selected. We have constructed a λ phage-based metagenomic library of the rumen protozoa using cDNA produced from protozoal RNA; this was then ligated into the λTriplEx2 vector and packaged into λ phage. The protocol was optimised to achieve a recombinant: non-recombinant phage ratio of >9:1 and insert sizes of >800 bps. Inserts (n=50) were amplified using vector-encoded primers and sequenced (ABI 3130xl), with most inserts confirming similarity to other protozoal sequences deposited in GenBank (Including Entodinium, Epidinium and Epispathidium. sp). Approximately 30,000 plaques were screened for cellulase activity using carboxymethyl cellulose as a substrate coupled with post-staining with Congo Red. Positive activity was identified in 5 plaques which are currently undergoing further characterisation. The library is also being screened for lipolytic and proteolytic enzymes using agar-based assays.

These findings provide further insight into the rumen eukaryotome whilst allowing discovery of enzymes which may be of use industrially.

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Single-Molecule observation of CRISPR-Cas9 dynamic behaviour in *Escherichia coli*

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CRISPR-Cas9, which is the popular enzymatic complex that produces DNA double-strand breaks when associated with a guide-RNA, has recently become a widely-used synthetic biology tool, allowing easy and efficient gene editing. However, the dynamics of this nucleo-proteic complex are not fully understood *in vivo*. For instance, we still do not know how long the protein remains on the DNA after the break, which could potentially have an impact on the DNA repair machinery, itself essential for any gene editing endeavour. Here, we propose to address this question in *Escherichia coli*, using single-molecule fluorescent microscopy in association with computational tracking to follow Cas9 behaviour over time, space and along DNA.

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Screening of growth conditions by UPLC-MS for novel Streptomyces secondary metabolite transcription

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Antimicrobial resistance has developed into one of the most dangerous global issues, predicted to cause 10 million deaths a year by 2050. New medicines are urgently needed, but drug discovery programmes have been largely abandoned due to low novel compound discovery rates. This is partially a result of the repetitive growth conditions used in the lab, which does not allow for the full range of natural environmental triggers that would usually stimulate secondary metabolite transcription. While this has resulted in a slow drug discovery pipeline, it also means the potential for new compounds from already known species is still high - if grown under the right conditions.

Culture collections such as NCIMB hold species isolated from around the world, including around 600 Streptomyces strains from locations such as a bee's intestines, the Mariana Trench and Atacama Desert.

Sets of soil, water and life-associated Streptomyces from the collection will be grown under a wide range of conditions, including different carbon sources, chemical elicitors, epigenetic inhibition and RNA Polymerase engineering. S. coelicolor A3(2) will be used for UPLC-MS optimisation to give the largest metabolite profile, after which the other species will be analysed to show which conditions produce novel natural products.

Preliminary results showed different morphologies and pigmentation from each of the proposed methods. More complete metabolite profiles will be built up for each species, creating polarity based chemical libraries with information on production of each compound.

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Mining the Human Microbiome for Bioactive Small Molecules

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Bacteria use small molecule chemicals to interact with each other and with their eukaryotic host. We use a combination of activity-driven and genomics-driven approaches to mine the human commensal microbiota for bioactive molecules, as well as the genes that encode their production. The ClusterFinder algorithm was developed to scan bacterial DNA for biosynthetic gene clusters (BGCs), identifying new classes of chemical compounds. Genetic and biochemical characterization of identified BGCs from skin bacteria revealed molecules with antibacterial, biofilm inducing and immunomodulatory functions. The identification of small molecule interactors produced by human commensals contributes to a better understanding of the complex interplay that is taking place in the communities from our microbiome. This will facilitate the development of therapeutic strategies to influence community structures with the aim to eliminate already established pathogens or inhibit their colonization prior to hospitalization.

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A spoonful of sugar helps the medicine go down: Production of recombinant human factor H in glyco-engineered yeast

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The alternative pathway of the complement system is regulated by the soluble 155-kDa glycoprotein complement factor H (CFH). An insufficiency of functional CFH increases the risk of age-related macular degeneration, afflicting 30 M people in the USA/Europe. Thus a recombinant (rec) source of CFH has potential therapeutic value. Therapeutically useful quantities of recCFH can be produced in Pichia pastoris. Unfortunately P. pastoris-produced glycoproteins have high-mannose N-glycans rendering them unsuitable for clinical applications.
This poster will describe "glycoengineering" P. pastoris for production of recCFH with human-type, complex N-glycans.

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Creating T4 DNA and RNA Ligase Mutants

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T4 DNA and RNA ligases repair breaks in DNA and RNA and also have many biotechnological applications as they are widely used in experiments that manipulate nucleic acids. There are two T4 RNA ligases – T4 RNA ligase 1 (T4Rnl1) and T4 RNA ligase 2 (T4Rnl2) and previous studies[1]have indicated that these ligases have a structural bias. Both enzymes are able to ligate combinations of double stranded DNA and RNA with varying rates of ligation, T4Rnl1 works on a wider range of substrates and T4Rnl2 has a more efficient rate of ligation[2]. To combat this bias new versions of the ligases were created by attaching the T4 ligases to other enzymes, resulting in fusion proteins that have dual functions. Adenylate Kinase (AK) is an enzyme that converts ADP to ATP and AMP. Importantly, ATP is the co-factor for ligation for T4 ligases. By creating AK-T4Rnl fusions we demonstrate that these proteins function not only as a ligase but also as a kinase, retaining function and efficiencies of both enzymes. The new AK-ligase proteins are able to create co-factor for the ligation reaction from ADP, potentially combatting some of the problems associated with co-factor degradation in the buffer.

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An Improved Synthetic Biology Toolbox for Developing *Clostridium* Species as Hosts for Biobutanol and Commodity Chemical Production

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The genus *Clostridium* contains multiple species that utilize carbohydrates via a bi-phasic fermentation. The end-products of the second phase (solventogenesis or Acetone-Butanol-Ethanol (ABE) Pathway) are of interest to industrial biotechnology. Improved yields and the ability to utilize complex substrates are important milestones toward achieving successful commercialization of the solventogenic *Clostridium* species. Genetic modification and synthetic biology can significantly help in the development of useful strains. However, the availability of biological parts in *Clostridium* is low and the knowledge of their precise biological activity is limited. We are developing a synthetic biology toolbox of transcriptional and translational regulatory elements for use in the solventogenic *Clostridium* species. Measurement of biological activity is achieved through the use of both novel (fluorescence) and established reporter (in *Clostridium*) systems. Fluorescence quantification is conducted using epifluorescence microscopy, microplate photometry and flow cytometry. We have developed a vector system that builds on existing ones by adapting to the MoClo Golden Gate assembly standard. The biological parts that we are developing adhere to that standard and provide an easy-to-use high throughput assembly and interchangeability of parts. As well as characterizing published parts, we are conducting bioinformatics-driven part-mining to discover and validate new parts. While this work has clear biotechnological goals and applications, we also aim to improve our understanding of regulatory element architecture in the genus and gain fundamental insight into gene expression determinants.

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Novel promoters for transgene expression in the model biofuel alga *Nannochloropsis gaditana*

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The marine microalgal species *Nannochloropsis gaditana* is able to accumulate high levels of lipids, including triacylglycerides (TAGs), which can be converted to biofuel through esterification. Additionally, *N. gaditana* has emerged as a platform strain for genetic engineering due to its ease of transformation and the availability of a fully sequenced and annotated genome. However, the development of molecular tools is imperative in fully realizing the biotechnological potential of this species. Endogenous promoters have been shown to be most effective for driving expression of transgenes in molecular engineering, however; only three endogenous promoters have been characterized in *N. gaditana* to date. Here we describe the establishment of an *in situ* green fluorescent protein (GFP) reporter system for the characterization of promoters in *N. gaditana* (CCMP526) and demonstrate its effectiveness by identifying and characterizing two novel endogenous promoters: a constitutively highly expressing promoter and an inducible promoter. Based on fluorescence and immunoblot analysis, both promoters showed promise for use in genetic engineering. The novel promoters and GFP reporter system described here will further enable the development of *N. gaditana* as a platform strain for genetic engineering for biofuel production and as a potential host for recombinant protein production.

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Does the DNA damage response depend on growth in bacteria?

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DNA damage events threaten the stability of bacterial lineages. To respond to such events, bacteria have developed sophisticated mechanisms. In particular, E. coli responds to DNA damage by activating the transcription of the SOS regulon which, in addition to inducing production of repair enzymes, can lead to increased mutagenesis and antibiotic persistence. Many of the SOS response variables are reliant on cell physiology, such as the frequency of DNA damage, and the capacity of cells to induce gene expression. The extent of these dependencies are largely unknown.
In this study, we explore how the response of E. coli to chronic double strand breaks depends on growth rate; by quantifying the distribution of SOS induction in single cells using fluorescent microscopy and microfluidics. We show that the variability of SOS response is inversely correlated with growth rate. Furthermore, under slow growing conditions, we observe a second population of highly induced cells which is absent at fast growth conditions. We discuss potential mechanisms involved in these phenomena, and their significance in the context of mutagenesis and antibiotic persistence.

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Modeling spatial efficiencies of large U.S. cities as a factor to the rise of canine MRSA cases

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A zoonotic infection with Methicillin Resistant Staphylococcus Aureus (MRSA) can be transmitted from canines to humans. Human spatial efficiencies such as centralized laundry network of major veterinary clinic and a network of dog parks alongside the failure of humans to practice aseptic techniques facilitates the rise of MRSA cases in select large U.S. metropolitan cities. The spatial efficiencies examined were the network of Banfield Pet Hospitals and city parks for each city studied. Part of this study looked at the impact of a contaminated laundry delivery vehicle for the Banfield network system and a dog park service use of contaminated cleaning equipment. This study use a routing software and topological maps and found that grid map layouts decrease travel time between Banfield Pet Hospital locations and improve dispersion of MRSA virus via the simulated delivery of contaminated veterinary technician's clothing. Cities that do not have planned dog parks such as Boston and Philadelphia have fewer formal interaction points between MRSA infected and non-infected canines and limits the spread of MRSA to other open park facilities. Cities with fewer dog parks and fewer Banfield Pet Hospital locations have lower formal dog to dog interaction nodes and points of dispersion via contaminated laundry. The outcomes of this study will assist in creating a temporal analysis on the days of the week that a canine MRSA outbreak may likely occur.

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A synthetic approach for the bioconversion of carbon dioxide to organic acid.

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Under anaerobic conditions using glucose as sole carbon and energy source, Escherichia coli can carry out a mixed-acid fermentation producing a range of organic acids (such as formate), ethanol, H2 and CO2. The key enzyme for H2 production is the formate hydrogenlyase (FHL) complex, which disproportionates formate into H2 and CO2. Although this reaction is the only one observed under physiological fermentative conditions, numerous studies suggest that the E. coli FHL complex can also perform the 'reverse' reaction. Using a combination of genetically engineered strains and optimized growth conditions, it has been unambiguously demonstrated that the FHL complex can operate in vivo and in vitro to fix CO2, paving the way for harnessing this enzyme in carbon capture projects and the generation of sustainable chemical feedstocks for industry. The main goal of this work is to improve CO2 conversion by E. coli. By using a bioprocess optimization approach, physico-chemical parameters governing this reaction (such as the H2/CO2 ratio, gas pressure and pH) were investigated in detail. Finally, further bioconversion of formate into other chemicals was also explored in this project. In keeping with the synthetic biology approaches already taken, a synthetic operon optimized for E. coli expression was designed that encoded NAD+/NADH-dependent formaldehyde and methanol dehydrogenases systems. The protein products were shown to be produced in an E. coli chassis and the ability of various engineered cells overproducing these enzymes to generate formaldehyde or methanol is being investigated.

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The Commensal Conspiracy: Can the microbiome enhance *Neisseria gonorrhoeae* colonisation?

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Pathogenic *Neisseria* species are able grow on the metabolic byproducts of other human microbiome members.
The *prp* gene cluster, present in both pathogenic *Neisseria* species (*N. gonorrhoeae* and *N. meningitidis*), is predicted to confer the ability to utilise propionic acid.
Co-occurrence between *prp*-containing *Neisseria* and *Porphyromonas* in the microbiome of the upper respiratory tract supports the hypothesis that propionic acid-producing bacteria increase the rate of colonisation by pathogenic Neisseria.
A similar interaction may occur between *N. gonorrhoeae* and a dysbiotic vaginal microbiome such as occurs in bacterial vaginosis (BV) characterised by several metabolites including propionic acid. Clinical studies indicate a link between gonorrhoea and BV, however this is the case for many STIs and so the mechanism is unclear.
With a well-defined *in vitro* co-culture assay that has strong correspondence with a mathematical model, *in vitro* data will be used for model fitting and quantification of metabolic and relational properties of the bacteria. The *in vitro* assay is a custom co-culture mini-bioreactor that gives tight control over growth conditions aiming for good correspondence to the mathematical model and on-line monitoring providing a wealth of data for model fitting. Quantification of bacterial properties is vital for clinical translation of findings using published metagenomic datasets and mass-spec analysis of the vaginal microbiome.
So far a systematic bacterial property quantification strategy has been developed through model simulation and analysis, this is being implemented *in vitro* with *N. meningitidis* and *Porphyromonas gingivalis*.
These findings will contribute towards the possibility of microbiome-based therapeutics for gonorrhoea.

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Plug and play - developing tuneable gene expression in microalgae using synthetic biology approaches

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There is enormous potential to use microalgae as feedstocks for everything from recombinant proteins and high value chemicals to biofuels, but to implement this technology in a sustainable and economic manner, it will be necessary to optimize many parameters, and metabolic engineering strategies will be essential. However, in comparison with the well-developed molecular biology approaches available for manipulation of bacteria, yeast, and even land plants, those for algae are limited, even for the well-studied *Chlamydomonas reinhardtii*. We have established a synthetic biology workflow to enable rapid assembly of different genetic elements (eg coding region, regulatory elements, targeting and epitope tags), allowing high throughput testing of different components, and ultimately orthogonality – where standard parts can be used in any system to generate predictable outcomes. Thiamine pyrophosphate (TPP) riboswitches are regions in messenger RNA that bind to TPP directly without the involvement of protein factors. Alternative splicing of the transcript then leads to changes in expression of the downstream open reading frame. Using our synthetic biology workflow we have investigated the mechanism of action of these sequences, and demonstrated that they can control transgene expression at nM concentrations of thiamine added to the cultures. By changing the aptamer sequence – the region that binds the TPP ligand – we can alter the sensitivity to different thiamine analogues. By this means we aim to generate a suite of riboswitches that can 'tune' expression of one or more transgenes.

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The Efficacy of a Next Generation Anti-Biofilm Complex on Biofilms and Biofilm-Associated Inflammation

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Aim: The use of a smart, trigger-release antimicrobial, anti-biofilm and anti-inflammatory complex is an urgent requirement in biofilm and wound management. The aim of this study was to investigate the efficacy of newly developed anti-biofilm complex on both planktonic microorganisms and biofilms.

Methods: *Staphylococcus aureus* and *P. aeruginosa* biofilms were grown for 48 hours using the MBEC and CDC bioreactor models. Biofilms were treated with the novel anti-biofilm complexes composed of anti-biofilm agents, antimicrobial agents and anti-inflammatory agents at different concentrations (200, 100, 50 and 25ppm). Anti-inflammatory capability of the complexes was evaluated in monolayer fibroblast scratch wound models and a 3-dimensional skin model *in vitro*, whereby the secretion of pro-inflammatory cytokines were monitored using ELISA kits. Direct and indirect cytotoxicity tests using assessed using L929 fibroblasts were performed to evaluate the cytotoxicity of the complexes.

Results: A Log-7 reduction of *S. aureus* and *P. aeruginosa* biofilms was observed following a 24-hour treatment. ELISA results showed significant changes in detectable secreted pro-inflammatory cytokines, including interleukin-6 and tumour necrosis factor-alpha, in both the monolayer scratch wound model and 3D skin model. Cytotoxicity studies identified a complex were that showed no cytotoxic effects in L929 fibroblasts cytotoxic.

Conclusions: The next generation smart and trigger-releasing anti-biofilm complexes in this study have demonstrated great potential for the management of biofilms and biofilm-associated inflammation. These complexes can be incorporated into an array of different platforms with significant benefit to the prevention and control of biofilms.

***Asepticate Plus**

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Aging of bacteria in biofilms, using an Individual-based model to study growth

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Aging has been defined as an accumulation of damage, or a loss of function, with increasing age. For bacteria, one strategy to cope with damage is asymmetric segregation of damage at division, so that the old pole cell inherits all of the damage, and the new pole cell is rejuvenated. Another, often neglected, mechanism is to repair the damage; our previous computational modeling work has found that an optimal rate of repair is fitter than damage segregation in well-mixed environments. Here, we investigate aging in biofilms, the predominant mode of growth for bacteria, with the individual-based model iDynoMiCS. We introduced adaptive repair, with and without damage segregation, to the previously used damage segregation and fixed optimal repair strategies. These are investigated in a constant environment, a chemostat, and in biofilms.

In the constant environment, symmetric division combined with fixed optimal repair was the fittest strategy. In the chemostat, symmetric division combined with adaptive repair was the fittest strategy. For biofilms, the fittest strategy depended upon the initial placement of cells. Where cells of competing strategies were placed in two side-by-side blocks, asymmetric damage segregation without repair was the fittest strategy. Where cells were placed in an alternating manner, the fittest strategy was symmetric optimal fixed repair.

Adaptive repair was not always the fittest strategy, as was expected, because the model did not allow protein previously allocated to repair machinery to be redistributed to growth machinery even when repair was no longer beneficial, therefore hindering growth.

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Production of Copper Peptide (GHK-Cu) as a Cosmetic Material in *Pichia pastoris.*

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Copper peptide is composed of three amino acids (Gly-His-Lys) and copper and naturally in human plasma. Since it was discovered in 1973, it has been reported that copper peptide plays wide roles in activation of wound healing, attraction of immune cells, antioxidant and anti-inflammatory effects, and stimulation of collagen synthesis. Especially, Copper peptide is widely used in anti-aging cosmetics. However, copper peptide has been produced by using chemical synthesis.

In this study, we developed a biological system to produce and purify this three peptide (Gly-His-Lys) by using a GRAS yeast, *Pichia pastoris*. First, we constructed an expression vector (pBJY-GHK6) in which six of Gly-His-Lys fused to His-taq could be expressed and secreted into media broth. The pBJY-GHK6 was transformed into *P. pastoris* and the recombinant strain was cultivated in minimal media (YNB/YSD without adenine). We used an affinity column (Ni-column) to purify the expressed cassette [$(His)_6$-K-$(GHK)_6$] and treated the expressed peptide with trypsin to generate GHK monomers. We confirmed the expressed cassette on SDS-PAGE and analyzed the GHK monomers by using HPLC and ESI-MS. We are developing optimized condition to increase concentration of the expressed GHK by optimization of culture media, fermentation mode and purification process. This system will be applied to widely used in production of cosmetic and cosmeceutical peptides.

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Genetic engineering of microorganisms for production of chemicals

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Ingenza is a synthetic biology/industrial biotechnology R&D company with a broad customer base across the chemicals, pharmaceuticals, food/feed and fuel industries. Our customers include (i) chemical companies looking to source capabilities in microbial/molecular biology and bioprocess development for the production of biobased chemicals and biofuels, (ii) therapeutics companies, looking to outsource the application of synthetic biology for natural product pathway and/or protein engineering/optimization and (ii) other SMEs/academics looking to transition early stage research through proof of concept and on to spinout/startup in human therapeutics. The Molecular Biology Department is responsible for the generation of unique, diverse, and complex strains capable of meeting various biosynthetic requirements. We aim to give an overview of the methodologies we use and highlight the power of combinatorial pathway assembly and screening.

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Impact of long-term heavy metals contamination on archived urban soil bacterial community structure

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Due to rapid industrialization and uncontrolled urbanization, soil contamination with heavy metals is a historical and continuing widespread problem, especially prominent in the vicinity of mines, smelters and industrial facilities. National geological surveys such as the British Geological Survey (BGS) provide a rich source of well-documented soil archives from regional, national and international locations. Our aim is to utilise the well-stocked archives of the BGS, especially the G-BASE collection, to determine, for the first time to our knowledge, microbial community compositions in archived soils, while also establishing the impact of metal pollution on archived soil community biodiversity and species richness. Although numerous studies have addressed the influence of exogenous pollutants on microorganisms, how long-term heavy metals exposure affects soil bacterial diversity was still unclear.

Topsoil samples (n=63) from Swansea-Neath Valleys (Wales, UK) were selected for analysis. The assessment of heavy metals pollution was derived using the geo-accumulation index (I*geo*). The thus calculated I*geo* pointed to moderate to high metals contamination in many of the archived soils.

Bacterial community was analysed using Illumina sequencing V3,4V 16S rDNA gene fragments amplified form soil-extracted DNA. At phylum level, the most polluted soil group (n=14) was dominated by *Proteobacteria*, whereas less polluted soils (n=49) were dominated by *Firmicutes*. *Proteobacteria* and *Firmicutes* were responsible for alpha diversity (average of similarity =45.40 and 31.71, respectively), with *Firmicutes* contributing 6.3% to beta-diversity. Spearman-analyses revealed significant correlations between four heavy metals and bacterial diversity ($p \leq 0.01$). Multivariate analyses showed that As, Cd and Zn significantly shaped bacterial community composition.

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Metagenomic and quantitative analysis of microbial communities involved in metal immobilization in freshwater sediments polluted with acid mine drainage

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Physicochemical- and microbial-mediated processes involved in metal retention in streams polluted with acid mine drainage (AMD) remain unclear. Two rivers have historically received AMD from the abandoned metal mine at Parys Mountain (Anglesey, UK). Sediment samples were obtained from both rivers and a wetland nearby the mine. Illumina next-generation sequencing of bacterial 16S rDNA was performed. Polymerase chain reaction of specific genes was conducted to measure bacterial activity related to metal immobilization. Metal levels were measured by ICP-AES. A total of 5 280 791 sequences and 103 300 operational taxonomy units were obtained. Bacterial diversity decreased in the most polluted sediments. The phylum Proteobacteria and the orders Methylophilales, Gallionellales and Xantomonadales were dominant in polluted sediments. Quantification of specific genes suggests that oxidation and subsequent immobilization of iron occurs in the upper oxic sediment of the wetland and which may explain the higher total iron concentration in surface sediment compared to the bottom layer. In contrast, concentrations of trace metals, in particular Cu and Zn, were higher in the anoxic bottom sediment compared to the surface. We conclude that migration of trace metals to the anoxic sediment occurs and that iron remains in the surface layers due to oxidation mediated by the dominant bacteria taxa.

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Degradation of volatile hydrocarbons in estuarine environments

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There is a gap in our understanding of the anaerobic degradation of isoprene and the non-methane gases especially ethane. This project seeks to understand the fate of five volatile hydrocarbons, namely isoprene, ethane, propane, benzene and toluene, due to microbial degradation in estuarine environments. Degradation was investigated in aerobic and anaerobic sediment slurry microcosms with samples collected at three locations along the Colne estuary in Essex, UK. Hydrocarbon loss due to degradation was monitored using gas chromatography coupled with flame ionisation detection. Significant aerobic degradation of each hydrocarbon occurred. Anaerobic degradation of toluene and benzene was observed, while isoprene, ethane and propane degradation was not seen even after 150 days' incubation. In aerobic microcosms, more than 80% benzene and toluene degradation was observed within six days and ten days for isoprene degradation. Ethane and propane degradation was generally slower and only about 60% degradation was observed with both carbon sources after 57 days. Analysis of the bacterial 16S rRNA gene sequences and denaturing gradient gel electrophoresis indicated that the main aerobic benzene-degrading bacteria were *Pseudomonas* spp. and the main toluene-degrading bacteria were *Amphritea* spp., while the isoprene-degrading communities were dominated by *Rhodococcus* spp. The ethane- and propane-degrading communities were a mixture of *Alpha-*, *Beta-* and *Gamma-proteobacteria*. Understanding the role of aerobic and anaerobic volatile hydrocarbon-degrading microbial communities will increase our knowledge of how these compounds are cycled in the marine environment.

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Microbial community composition of hypersaline microbial mats from the Abu Dhabi sabkha Microbial community composition of hypersaline microbial mats from the Abu Dhabi sabkha

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Microbial mats are organo-sedimentary structures found in areas with extreme environmental conditions. Since these ecosystems are considered the oldest forms of life on Earth, the study of microbial mats can inform our understanding of the development of life early in the history of our planet. In this research, we used hypersaline microbial mat samples from the Abu Dhabi sabkha (salt flats in Arabic) to study their contribution in source rock formation. Syringe cores of about 90 mm were sampled from an intertidal area and total genomic DNA extracted from 2-3 mm thick sub-sections along the depth of the core. Individual DNA extracts were analysed via Ion Torrent 16S rRNA amplicon sequencing to investigate the composition of the microbial community along the studied cores. Preliminary results show high proportion of *Archaea* in all layers, with *Halobacteria* appearing to be more significant in the first 40 mm. Members of the class *Deltaproteobacteria* are distributed vertically with significant abundance (10-20%), but less numerous in the first 8 mm of the mat, where *Chloroflexi* and *Anaerolinea*, both green non-sulfur bacteria, are predominant. *Cyanobacteria* was found only in the top 10 mm, with unexpected low abundance (less than 3%). These results show a vertical zonation along the microbial mat sample from one site of the transect. Further analyses will be done to investigate if these patterns repeat in other sites along the transect, and to relate the microbial composition to the physico-chemical conditions of the sites.

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Microbial degradation of cellulose and its alkali hydrolysis products; implications for radwaste geodisposal

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Intermediate-level radioactive waste from the nuclear fuel cycle, which is expected to contain cellulosic material encapsulated in cement, will be disposed of via a deep geological disposal facility (GDF). Under the hyperalkaline conditions imposed by the resaturation of cement with groundwater, cellulose will be chemically hydrolysed to short chain organic acids. The most abundant hydrolysis product is isosaccharinic acid (ISA), which has been shown to bind to and mobilise various radionuclides, thereby increasing the probability of their release from the GDF. However, alkaliphilic microorganisms may survive in such extreme environments potentially using these organics as a carbon and energy source.

Microcosms poised at pH 12.2, and inoculated using sediments from a legacy lime-workings, showed biodegradation of added cellulose and fermentation of the degradation products into acetate, while halting ISA production from abiotic cellulose hydrolysis. Enrichment cultures prepared at pH 10 and inoculated with sediments from the same site showed that alkaliphilic bacteria degrade ISA under aerobic and anaerobic conditions. An ISA-oxidising obligate alkaliphile belonging to the genus *Anaerobacillus* was isolated from these cultures, and was found to precipitate radionuclides, including soluble U(VI) from solution. Comparative genome and transcriptome analysis, coupled to transmission electron microscopy and X-ray absorption spectroscopy are helping identify the mechanisms of ISA degradation and radionuclide precipitation by this novel bacterium. Taken together, these results highlight the role that microorganisms may play in stabilising radioactive waste in the subsurface, and help reduce uncertainties in the long-term performance assessment of the GDF for radioactive wastes.

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Proteomic insights into anoxygenic phototrophic iron(II) oxidation by *Rhodopseudomonas palustris* TIE-1

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Photoferrotrophy, the oxidation of Fe(II) by anoxygenic photosynthetic bacteria, has been suggested to have supported Earth's biosphere prior to the evolution of oxygenic photosynthesis and still has an important role in the biogeochemistry of modern environments. All known organisms which can perform photoferrotrophy show remarkable metabolic flexibility and can utilize a wide range of organic and inorganic substrates, yet the cellular adaptations required to oxidize Fe(II) are not well constrained. In this study, we use quantitative proteomics to compare the proteome profiles of the photoferrotroph *Rhodopseudomonas palustris* TIE-1 grown phototrophically with either Fe(II), hydrogen or acetate. We have identified more than 180 proteins which are present only during Fe(II) oxidation. These indicate that photoferrotrophy i) causes changes in the cell structure including increased synthesis of peptidoglycan, ii) induces the formation of extracellular structures such as flagella, and iii) requires increased DNA repair. In addition, changes in carbon metabolism, light harvesting processes and management of redox homeostasis are detected which appear to be general responses to photoautotrophic growth (i.e. present with both Fe(II) and H2). The presence of Fe(II) also inhibits proteins which import Fe-complexing siderophores despite the fact that this strain does not produce siderophores under any of the growth conditions tested. Additionally, our results are consistent with an observed role for the pio operon in Fe(II) oxidation in this organism, and show that only PioC, a high potential iron-sulfur protein, is unique to growth on iron. This work provides novel insights into the wider cellular adaptations required for photoferrotrophy.

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Methanethiol-dependent dimethylsulfide production in soil environments

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Dimethylsulfide (DMS) is an environmentally important trace gas with roles in sulfur cycling, signalling to higher organisms and atmospheric chemistry. DMS is believed to be predominantly produced in marine environments via microbial degradation of dimethylsulfoniopropionate (DMSP). However, significant amounts of DMS are also generated from terrestrial environments, likely via the methylation of methanethiol (MeSH). We identified a methyltransferase termed "MddA" that likely mediate this process. MddA functions in a wide range of bacteria and some cyanobacteria. The *mddA* gene is present in metagenomes from varied environments, being particularly abundant in soils, where it can be present in up to 76% of bacteria. These results suggested that the MeSH-dependent DMS production (Mdd) pathway may significantly contribute to global DMS emissions, especially in terrestrial environments. To further confirm this hypothesis, we investigated the functionality of the Mdd pathway in a wide range of environments. All terrestrial environments tested produced DMS from MeSH. Cultivation-dependent and cultivation-independent methods were used to assess changes in bacterial populations in these environments upon addition of MeSH. Bacteria of the genus *Methylotenera* were enriched in the presence of MeSH. Furthermore, many Mdd^+ strains were isolated but did not contain *mddA*, likely indicating metabolic flexibility in the Mdd pathway.

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Geochemical influence on Se/Te oxyanion reduction by yeasts

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Selenium and tellurium are two metalloids critical to environmental technologies. In addition selenium and tellurium oxyanions can be toxic in the environment and can affect human health. This work aims to examine geochemical influence on Se/Te reduction, using a simple yeast model system, to identify what limitations there are to the process, and their importance. Several yeast strains, capable of selenite or tellurite reduction, were isolated from environmental samples. Growth was assessed in the presence of selenite or tellurite and minimum inhibitory concentrations determined. The production of elemental Se^0 or Te^0 was analysed using energy dispersion X-ray analysis (EDXA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). This work aims to increase knowledge of selenium and tellurium cycles and to investigate potential routes of Se/Te biorecovery.

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Bioprospecting for novel antimicrobial drugs from microbes in glacial environments

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Given the alarming levels of global antimicrobial resistance, there is an urgent need for novel antimicrobial drugs. Extreme ecosystems such as glaciers are a poorly developed resource for antimicrobial discovery. Glaciers make up ~10% of the Earth's surface and contain diverse microbial life. Given the harsh conditions these microbes experience it is thought they may produce stress response compounds which could possess antimicrobial properties.

A combination of culture-dependent and culture independent methods are being used in our search for drug leads. Our pilot studies reveal isolates demonstrating antimicrobial activity against the important human pathogen *Pseudomonas aeruginosa*. Subsequently, a large isolate library is being developed from glacier samples by optimising growth conditions: investigating effects of temperature, enrichment broth and media to improve isolation and cultivation of glacier microbes. However, we are also applying sequence-based metagenomics approaches to glacier samples which will be complemented by activity based functional metagenomic screening using Fosmid vectors.

The most potent isolates will be selected for downstream chemical analysis to purify and identify the bioactive compounds. Purified compounds may undergo cytotoxic testing and further safety assessments to verify their potential as novel antimicrobial drugs.

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The microbial ecology and biogeochemistry of a nuclear fuel storage pond

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The Sellafield site has several open spent nuclear storage ponds (SNFP) that are at various stages of decommissioning, including an alkaline pond containing spent fuel and sludge waste, which are to be retrieved. This pond is extremely inhospitable with significant levels of radioactivity and high pH (~11) due to the continuous purging of highly alkaline water. Despite these conditions the pond is known to experience seasonal microbial growth, described as "algal blooms". The blooms reduce visibility in the pond, impacting on waste retrieval operations and potentially increasing both the cost and timescale of hazard and risk reduction. Currently there is little information about the organisms responsible for the blooms, what the triggers for the bloom are, or how the microorganisms colonise such an extreme environment.
The aim of this project is to characterise the microbial community residing in this pond. Initial analyses of DNA extracted from the pond water samples revealed the presence of a cyanobacterium, Pseudanabaena catenata. Cultures of P. catenata were exposed to 95 Gy of X-irradiation over a 5 day period to monitor the effect that ionizing radiation had on the growth of the organism. Cell pellets were analysed using FTIR to get a fingerprint of the metabolic activity in the irradiated cells. P. catenata showed no change in growth yield when irradiated. However the cultures did show a significant reduction in the chlorophyll concentrations following the treatment. FTIR results indicate an increase in polysaccharides, which may significantly impact on the fate of radionuclides in the pond.

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Investigating the potential application of Microbial Enhanced Oil Recovery on unconventional oil: A field specific approach

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A substantial amount of the world's recoverable oil reserves are comprised of unconventional resources, however great difficulty has been had in recovering this oil. Therefore, advanced methods such as microbial enhanced oil recovery (MEOR) have been employed. MEOR involves the use of bacteria and their metabolic products to alter the oil properties or rock permeability within a reservoir in order to promote the flow of oil. Although MEOR has been trialed in the past with mixed outcomes, its feasibility on heavier oils has not been fully demonstrated. The aim of this study was to show that MEOR can be successfully applied to unconventional oil fields to increase oil production. Using both genomic and microbiologically applied petroleum engineering techniques, we have identified key mechanisms responsible for driving the promotion of oil recovery in heavy oil fields. By identifying key microorganisms with MEOR potential, using a new pipeline for 16S analysis, we were able to target and culture an indigenous bacterial isolate from the reservoir of interest. This strain was applied to field specific microcosms and the effect of this microorganism was compared to variant inocula, showing recoveries of ~ 12% additional oil recovery, beyond levels shown by previous MEOR related bacteria. Comparative genomics also revealed key genetic alterations between this and similar MEOR strains that could hold the key to its increased potential for future MEOR strategies. By altering these genes, we are able to identify which are responsible driving factors behind the oil/bacterial/rockface interactions.

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The biogeography and survival of haloarchaea inside halite crystals

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Haloarchaea (order Halobacteriales) dominate in a variety of oxic hypersaline environments such as solar salterns and salt lakes, and are even found in ancient salt deposits. Analysis of haloarchaeal 16S rRNA gene sequence data derived from halite crystal samples taken from artificial solar salterns in locations across Western Europe, the Mediterranean and around Madagascar showed some regional differences, such as a significantly greater abundance of *Haloquadratum* spp. in salt crystals from the Mediterranean. In addition, there were some abundant and widely distributed genera such as *Halobacterium* and *Halolamina*. In order to further understand which haloarchaea preferentially survive entombment in halite, using a 16S rRNA DGGE and Illumina amplicon approaches, we will compare the in-situ community composition of brines and crystals taken from Trapani salterns (Sicily), and will also identify successional changes in microbial communities derived from Trapani saltern brines after entombment in halite. Results from these and further experiments may unearth novel survival strategies that could inform the search for extra-terrestrial microbial life and allow a better understanding of long-term survival.

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Biomineralization of lanthanum by *Aspergillus niger*

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Occurring with associated minerals in the Earth's crust, lanthanum is one of the most important members of the lanthanides that share similar physicochemical properties and have a variety of industrial applications. *Aspergillus niger* is widely used in industrial fermentations due to its production of multiple secondary metabolites including enzymes and organic acids. It is a ubiquitous soil inhabitant, and since it produces geoactive agents such as citric and oxalic acid, it can play a role in the biotransformation of metal-containing minerals. The aim of this project was to explore the mechanisms and factors involved in the interactions between lanthanum and oxalate-producing strains of *A. niger*. As shown in previous studies, *A. niger* has significant properties of mineral solubilization and secondary mineral formation, many of them being precipitated as metal oxalates. However, there is little or no information on the possible formation of lanthanum oxalates mediated by fungi. In this study, fungal growth under conditions of lanthanum stress was investigated, and it was found that crystalline deposits were formed around fungal colonies in the presence of $LaCl_3$. The biogenic crystals were recovered and subjected to examination for their elemental composition, morphological features and mineral phases using energy dispersive X-ray analysis (EDXA), scanning electron microscopy (SEM) and X-ray crystallography (XRD) respectively. These confirmed the production of lanthanum oxalate ($La_2(C_2O_4)_3 \cdot xH_2O$). This work sheds light on lanthanum-*A. niger* interactions and potential applications in biorecovery of this precious element.

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The fate of nickel during isosaccharinic acid (ISA) biodegradation in nuclear waste disposal

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In the UK the concept to dispose of radioactive High-level (HLW), spent fuel and Intermediate-level wastes (ILW) is via a cementitious geological disposal facility (GDF). In a cementitious disposal concept upon closure, the facility is expected to resaturate with groundwater, creating a hyperalkaline plume that will lead to degradation processes. Susbtantial amounts of cellulosic items present in ILW will degrade, resulting in the production of small organics, of which isosaccharinic acid (ISA) will be the most dominant. ISA is a stable, organic molecule that forms water-soluble complexes with radionuclides, including radioactive nickel (Ni), present in the wasteforms and thereby might enhance radionuclide solubility and thus transport.

Since ISA is an organic substrate that may be used for microbial metabolism, the role of microorganisms in moderating the transport of Ni-ISA complexes from a GDF was investigated in this study. We report the fate of Ni complexed to ISA in circumneutral microcosms poised under anaerobic conditions; with and without Fe(III) added as electron acceptor for microbial metabolism. Data presented, confirm metabolism of ISA to acetate and butyrate, prior to utilization of these acids during Fe(III) reduction. The fate of Ni was identified with ICP-AES. XRD, TEM and ESEM analyses were used to obtain insight into the impact of biomineral formation in controling the the mobility of Ni. Also presented are the microbial communities associated with these processes, characterized using next generation 16S rRNA gene sequencing. Finally, the impact of ISA degradation on radionuclide solubility, and hence mobility, will be discussed in the context of geological disposal in radioactive waste.

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Microbial Community Evolution is Significantly Impacted by the use of Calcium Isosaccharinic Acid as an Analogue for the Products of Alkaline Cellulose Degradation

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Under anaerobic, alkaline conditions cellulose hydrolyses to a range of small molecular weight compounds collectively known as cellulose degradation products (CDP). CDP has received considerable attention since its generation within geological disposal sites for intermediate level radioactive waste (ILW) is expected to enhance radionuclide transport through complexation reactions. The microbial degradation of CDP has the potential to counteract this impact through the removal of the complexants concerned. CDP contains a range of saccharinic acids capable of complexation, however the Calcium salt of α-Iso sacharinic acid (α-ISA) is commonly used as an analogue for CDP. In this study, the impact of substituting α-ISA for CDP on the evolution of a polymicrobial alkaliphilic consortia derived from a hyperalkaline soil was investigated. In both cases the overall pattern of metabolism was similar, with ISA degradation being dominated by acetogenesis and hydrogenotrophic methanogenesis. Whilst there were no overall differences in the degradation profiles of either α-ISA or the range of ISAs in CDP significant differences ($p = 0.004$) were seen in the associated microbial populations. For example members of the genus *Acetovibrio* were more prevalent in the CDP fed communities as were members of the genus AB218327 which showed greatest sequence homology to *Anaerotruncus colihominis.* The study demonstrates that α-ISA is not an appropriate analogue for CDP in microbial ecology studies of the evolution of GDF for ILW since it generates a significantly different microbial community structure.

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Tellurium and Selenium Cycling and Supply

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Microbial Se or Te reduction offers a potential route to biorecovery of these elements from solution. Reduction is often efficient and large amounts of these metalloids can be removed from solution, resulting in extensive precipitation around biomass. This is more effective than biomethylation, which can result in only small amounts of removal, and would necessitate a further trapping step to recover volatilized methylated derivatives. Therefore, microbial reduction of Se or Te oxyanions from Se/Te-loaded solutions or leachates potentially provides a novel means for effective recovery of Se and Te from aqueous solution. Experimental work has examined fungal growth and bioreduction of Se and Te oxyanions in culture media. Fungi capable of Se/Te reduction were isolated and tested for tolerance to Se/Te oxyanions and efficient Se and Te-reducing fungal strains have been identified. Reduction efficiency, and growth responses to Se/Te, were determined by biomass yield and radial growth assays on Se/Te-containing media. Biotransformation of Se/Te by selected fungal strains was confirmed by resultant colour changes in solid and liquid media, and supported by light and electron microscopy coupled with energy-dispersive X-ray analysis.

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Characterisation of methylotrophs in the rhizosphere through DNA and RNA Stable Isotope Probing

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Methanol is an abundant volatile organic compound with an important role in atmospheric chemistry. The large disparity between methanol production by plants and methanol emissions to the atmosphere is hypothesised to be due to uptake by plant-associated methylotrophs, microorganisms capable of one-carbon metabolism. To investigate plant exudate-utilising methylotrophs in the rhizosphere, DNA and RNA stable isotope probing (SIP) experiments were performed by cultivating pea and wheat plants in a 13CO2-rich atmosphere, consequently labelling exudate-consuming microbes. The bacterial rhizosphere community, which was successfully labelled with plant-derived 13C, was characterised by metabolic and 16S rRNA gene sequencing using the labelled DNA and RNA extracted from the rhizosphere soil. Methylotrophs in the rhizosphere of pea and wheat were further investigated by DNA-SIP using 13C methanol, with shotgun metagenomics of the labelled DNA. We identified distinct methanol-utilising communities associated with each plant species, in comparison with unplanted control soils. These data, together with characterisation and genome sequencing of isolates, help to reveal patterns of methylotrophy among plant-associated bacteria. This study, using both cultivation-dependant and cultivation-independent techniques, advances our understanding of the bacterial utilisation of plant exudates and the role of methylotrophy in the rhizosphere.

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Getting metal out of stone; microbial mining of lateritic cobalt & nickel at near-neutral pH

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Cobalt is an essential element for modern technology, and critical for a low carbon economy. The most recently developed cobalt resource is a by-product from the mining of Fe-rich nickel laterites developed on deeply weathered ultramafic rock. Here we describe the fate of cobalt and nickel during the development of microbially-reducing conditions at near-neutral pH.

Three lateritic sediments were examined; two fossil laterites (Çaldağ, Turkey and Shevchenko, Kazakhstan), and one actively-forming laterite (Acoje, Philippines). Goethite was the predominant iron-containing mineral phase and the laterites contained 20-45wt% Fe, 1.4-1.7%wt Ni, 0.12-0.2wt% Co and 0.4-1.2wt% Mn. Sequential extraction showed the majority of Co and Mn were present in the reducible fraction, while the Ni and Fe were largely associated with the recalcitrant residual fraction.

Sediment microcosms were set up for each laterite with acetate added as an electron donor to stimulate the natural microbial community and the development of microbially-reducing conditions. Parallel microcosms were set up in a bicarbonate buffer with acetate and inoculated with *Geobacter sulfurreducens* to enhance iron(III)-reduction.

The microcosms with *G. sulfurreducens* rapidly generated considerable quantities of Fe(II) associated with the solid phase. Sequential extractions found a significant quantity of metals had been shifted into the exchangeable ‘sorbed' fraction after biostimulation, and could be solubilised by extraction with acetic acid. $L_{2,3}$-edge XAS indicated that the majority of the iron remained present as Fe(III), despite distinct morphological changes being observed. These results suggest that stimulating bioreduction processes shows potential for extracting cobalt and nickel from laterites.

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Genomic clues of adaptation to deep life

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The Fennoscandian Shield in Finland dates back to the Archaean and Proterozoic eons, and hosts ancient fluids (> 50 million year old) and thriving microbial life, but little is known about the limits of life in this harsh environment. Our project aims to explore the adaptability and metabolic potential of the deep biosphere by defining the features that allow microbes to survive in these extreme habitats and estimating the possibility of the emergence, development and sustainability of life in nutrient-limited and anaerobic deep subsurface settings. We utilized 16S rRNA gene amplicon sequencing to characterize the microbial community structure from deep, anoxic, saline groundwater originating from ~1.93–1.92 Ga old Fennoscandian granitic basement from Pyhäsalmi mine. A major component of the bacterial community, representing over 65% of the sequences, was a phylotype affiliating with *Alkanindiges*. The type species of this genus, *A. illinoisensis*, is hydrocarbonoclastic and uses straight-chain alkanes such as hexa- and heptadecane as a substrate. The phylotypes of the archaeal community were affiliating with *Methanobrevibacter* and thaumarchaeotal Candidatus *Nitrosopumilus.* Thaumarchaeota consist of ammonia-oxidizing archaeal groups, physiologically adapted to low energy supply and an oligotrophic lifestyle, adaptations highly relevant to deep terrestrial subsurface inhabitants. In addition, a novel variant of an autotrophic CO_2-fixation pathway has been discovered from *Nitrosopumilus* archaeon, making these archaea especially interesting when defining the carbon cycle in deep subsurface environments. We will use metagenomics analysis to further probe these intriguing microbial communities in order to shed light to their metabolic capacities and functionality.

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Dispersant impacts on microbial activities and oil biodegradation potential in the Arctic Ocean

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Oil tanker and pipeline spills are catastrophic for all affected ecosystems. In addition, certain spill response strategies, particularly the application of chemical dispersants, may also have harmful effects. During the Deepwater Horizon oil spill in the Gulf of Mexico, for example, around 7 million litres of chemical dispersants were applied. However, the impact of these chemicals on native microbial communities remains unclear. Particularly pristine, extreme environments like the Arctic Ocean are largely unexplored, while pollution risks are increasing due to the rising interest in arctic oil exploration and transportation routes. The aim of this study was (1) to assess the impacts of dispersants on microbial activities in arctic seawater and (2) to study oil biodegradation potential, with respect to the influence of dispersants, nutrients, and temperature. For this, a Deepwater Horizon-like spill scenario was simulated in laboratory microcosms with 150 m deep arctic seawater amended with oil only (supplied as water-accommodated fractions; oil-WAFs), oil-dispersant mixtures (chemically enhanced WAFs; CEWAFs), only dispersant (dispersant-WAFs), or oil-WAFs with nutrients. Microcosms were incubated at 1.5 °C or 15 °C in the dark and sampled after 0, 5, 12 and 32 days. Marine oil snow formation was observed in oil-WAF (± nutrients) and much less in CEWAF microcosms. Additionally, all hydrocarbon-exposed microcosms showed comparable DOC contents on day 0 but only oil-WAFs showed a substantial decrease during the experiment. Furthermore, analysis of ^{14}C-hydrocarbon oxidation rates, ^{3}H-leucine-based bacterial productivity, and cell counts will help elucidate the impacts of dispersants on microbial communities from the Arctic Ocean.

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Understanding the microbial productivity in highly radioactive storage facilities

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The use of nuclear energy has been of great importance to the United Kingdom, with Sellafield being the largest nuclear site used for both power production and more recently reprocessing activities. This project, via collaboration between the Geomicrobiology Group at the University of Manchester and Sellafield Limited, aims to investigate the biogeochemical conditions of a Fuel Handling Pond (FHP) in Sellafield.

Samples were taken from the FHP at different spatial locations and depths, encompassing subponds and channels. First, microbiological techniques are used to count and isolate culturable representative of the microbial community of the pond system (FHP). Complementary DNA-based techniques were also used to give more comprehensive view of the indigenous microorganisms, based around qPCR and Illumina sequencing of the 16S rRNA genes. Preliminary culture dependent and DNA profiling data will be presented.

Future plans involve further genetic characterisation of the identified communities which will be correlated with the complex biogeochemistry of the pond system. These studies will lead to a better understanding of the factors supporting colonization of the pond, the potential impact of the microbial community on fuel handling operation and the biochemical mechanisms of adaptation to this extreme radioactive environment.

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Geobiotechnological approach to evaluate bacteria as proxies for paleo sea-level reconstruction

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Background
Although microbes account for 90% marine biomass, tracing bacteria for paleo sea-level reconstruction remains unexplored. Unveiling bacterial diversity in beach ridges through metagenomic approach and bacterial microenvironment annotation throw insights into tracing paleo sea-levels.

Methods
Sediment core was excavated from a beach ridge at Vettaikaraniruppu (VKI; N 10° 33.208’ E 79° 50.127’; 2.6Km inland), Cauvery delta, Tamil Nadu, India. Subsamples were retrieved from top (VKIT: 2.8m), middle (VKIM: 7.2m) and bottom (VKIB: 24.5m). Bacterial diversity was assessed through V3 region targeted 16SrDNA metagenomic analysis. Micropalaeontological studies and Optically Stimulated Luminescence (OSL) Dating were performed.

Results
Metagenomic analysis revealed 38 bacterial phyla totally. 15 phyla were exclusive for VKIM; 1 phylum for VKI-B and none for VKI-T. Species diversity showed 701 species in VKIM, 588 in VKIT and 472 in VKIB. Habitat annotation revealed 29 exclusive marine bacterial genera in VKI¬-M; 16 for VKI-T and VKI-B, depicting 81% increase of marine bacterial genera in VKIM when compared to VKIT and VKIB. Foraminifera were detected only at VKI-M. OSL chronology revealed VKIB as late Pleistocene (146.64 ± 36.81 Ka BP); VKIM and VKIB as Holocene epoch (6.04 ± 1.25 and 3.36 ± 0.42 Ka BP respectively).

Conclusion
The highest bacterial diversity and presence of abundant marine bacteria indicated that VKI-M was marine environment at 6.04 ± 1.25 Ka BP, which was confirmed by the presence of foraminifera. Ratio of abundant to rare marine bacterial genera at various depths of VKI illustrated the prospects of tracing paleo sea-levels based on bacterial proxies.

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Paper title: Isolation and characterization of novel alkaliphilic, bacteria from hyper-alkaline, Lime contaminated soils.

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The alkaliphiles represent a group of organisms whose metabolic processes may be utilised for novel industrial and bioremediation applications. In addition, the survival mechanisms employed, particularly in calcium dominated alkaline environments are poorly understood. Examples of Ca^{2+} dominated alkaline environs exist as a result of the re-saturation of lime wastes following historical depositions into valley faces, such as Harpur Hill, Buxton, UK. Within these environments, the porewater pH is >13, resulting in the anaerobic, alkaline degradation of organic materials at depth. The sub-culture of these alkaline soils within microcosms using synthetic alkaline cellulose degradation products resulted in the isolation of a catalogue of micro-organisms. These species were identified using 16S rRNA, and found to belong to the Proteobacteria, Bacteroidetes and Firmicutes. pH tolerance was determined using BioscreenC technology and used as a selection criteria for further whole genome sequencing via NGS. The resulting sequences provided information with respect to metabolic capabilities and survival strategies. These were coupled to examination of phenotypes, employing analysis of cellular fatty acids, biofilm production, metabolic profiling and tolerance to metals. Overall, the study provides a range of characterised isolates that provide a platform for industrially applicable investigations.

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Ecophysiological adaptations of the weathered shale isolate *Variovorax paradoxus* HW1

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Geological deposits such as shale rock hold one of the largest reservoirs of organic matter (OM) on Earth, more than is contained within the oceans or the biosphere. The weathering and release of this OM at the Earth's surface has a significant impact on the global carbon cycle, yet the contribution of biological activity to these weathering processes is poorly constrained. Previous studies have focused on microbial communities that grow using shale OM has a sole carbon source, but few have examined how individual organisms interact with their geological environment.

Shale and coal are both OM bearing sedimentary rocks, but they differ in their OM chemical composition and structure. The effect of these rock types on the growth and motility of the weathered shale isolate *Variovorax paradoxus* HW1 in a minimal salts medium either with or without an additional bioavailable carbon source was investigated.

Shale rock powder stimulated both the growth and swarming motility of HW1, with greater growth stimulation observed in medium lacking an additional carbon source. Coal in contrast appeared to have an inhibitive effect on growth in the absence of a carbon source. Evidence that coal induces a physiological stress response in HW1 included behavioural changes in colony morphology and in swarming motility. Further experiments have revealed that HW1 has complex physiological responses to alternative carbon sources including naphthalene, casamino acids and rubber.

These results demonstrate that *V. paradoxus* HW1 is well adapted to growth and survival in OM rich geological environments.

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Global meta-analysis reveals the terrestrial deep subsurface microbiome is defined by host-rock-specificity and a core of pivotal taxa

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Microbial communities across the terrestrial deep subsurface have been studied more intensively in recent years as they are thought to represent 2-19% of all biomass across the Earth. However, while concentrated efforts have been made towards characterizing specific sites and regions, large-scale trends of microbial communities inhabiting these settings have yet to be investigated. Here, a collection of publicly available amplicon datasets targeting the terrestrial deep subsurface have been analysed using a closed-reference OTU picking approach. As such, a direct comparison of samples of diverse geological and geographical origins across the Earth's crust was made possible for the first time.
Strong evidence towards host-rock-specificity has been noted across a number of rock types, unexpectedly in spite of depth. Phylum- and class-level differential abundances suggest local specialization patterns in the function of local settings defined by host-rock chemistry. Nonetheless, terrestrial subsurface bacterial and archaeal communities seem to share a number of OTUs central to the overall community structure. A ‘deep terrestrial core' of microbes may thus be essential for the establishment of microbial communities in the deep subsurface. Specifically, Pseudomonas made up half the set of OTUs composing the most central taxa to this ‘deep terrestrial core', supporting the hypothesis that this is a keystone taxon in subsurface environments. The genomic and metabolic plasticity of this genus may underpin its function within deep subsurface microbial communities.
This work provides the first large-scale evidence for host-rock-specificity as well as for a small pivotal cohort of OTUs central to deep subsurface microbial communities.

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Biofilms Confer Resistance to Simulated Extra-terrestrial Geochemical Extremes

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Biofilms improve microbes' resistance to a variety of extreme conditions but little is known about biofilms influence under extra-terrestrial conditions, which is of interest due to the discovery of putative aqueous environments on other planetary bodies including Mars.

We investigated the loss of viability of planktonic cells and biofilms of Sphingomonas desiccabilis in simulated martian brines. These brines were produced from geochemical modelling of past aqueous environments on Mars and present a unique test of microbial viability as they are fundamentally different from terrestrial brines due to their high sulfate content.

Biofilms grown on basaltic scoria were subjected to the martian brines and the viability of cells was measured over time and compared to equivalent planktonic cultures. Crystal violet assay was used to measure how the biomass of the biofilms changed over time in response to the brines.

We found that biofilms that were desiccated prior to being treated with otherwise hostile brines maintained viability over a longer treatment duration compared to planktonic cells. Biofilm biomass increased after a few hours even in very harsh brines, suggesting cells were responding to stressful conditions by producing more EPS.

Our results show that biofilms confer protection to the harsh conditions of these brines, which have relevance to rare environments on Earth (e.g. the Basque lakes of British Columbia) and extra-terrestrial environments such as Mars. This has implications for the possibility of life in these environments, the detection of such life by future missions, and planetary protection considerations when sending missions there.

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The Janus face of Iron on anoxic worlds: Iron oxides are both protective and destructive to life on the Early Earth and present-day Mars

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The surface of the early Earth was probably subjected to a higher flux of ultraviolet (UV) radiation than today. UV radiation is known to severely damage DNA and other key molecules of life. Using a liquid culture and a rock analogue system we investigated the interplay of protective and deleterious effects of iron oxides under UV radiation on the viability of the model organism, *Bacillus subtilis*. In the presence of hydrogen peroxide there exists a fine balance between iron oxide's protective effects against this radiation and its deleterious effects caused by Photo-Fenton reactions. The maximum damage was caused by a concentration of hematite of ~1 mg/mL. Concentrations above this confer increasing protection by physical blockage of the UV radiation, concentrations below this cause less effective UV radiation blockage, but also a correspondingly less effective Photo-Fenton reaction, providing an overall advantage. These results show that on anoxic worlds, surface habitability under a high UV flux leaves life precariously poised between the beneficial and deleterious effects of iron oxides. These results have relevance to the early Earth, but also the habitability of the surface of Mars, where high levels of UV radiation in combination with iron oxides and hydrogen peroxide can be found.

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The impact of salt composition on communities of entombed halophilic micro-organisms

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Halophilic (salt loving) microorganisms are able to entomb themselves within the fluid inclusions of salt crystals for geologically-long periods of time. The mechanism behind this process is still unknown, and it is unclear whether the cells remain metabolically active. It is also unknown whether the cationic composition of salts can affect the composition of the entombed microbial community.
Samples of halite (NaCl), potash (KCl and NaCl in equal concentration) and the transition between the two were collected from Boulby Mine, UK. Geochemistry and mineralogy were determined using ICP-MS and XRD. The microbial community within the different salts was characterised using a combination of culture independent TRFLP and Illumina sequencing. The geochemistry was compared to the molecular biology data using ANOSIMS statistical tests and plotted on NMDS graphs.
There was no detectable difference in the diversity of the microbial community across the large ranges of Na and K concentration observed (15-40% and 0-30% respectively). The bacterial community, however, was significantly different across a narrow range of Ca concentrations (0-3%), although the archaeal community remained consistent.
XRD showed that Ca was predominantly present in CaSO4, which implies fluid flow through the salts and secondary mineral precipitation. Therefore, it could be suggested that aqueous alteration has impacted the bacterial community, but not the archaeal community. Further investigation is needed to determine whether the bacterial differences result from changes in local chemistry resulting from an influx of fluid, or whether fluid flow has facilitated transport of bacteria from elsewhere in the geological column.

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Can we catch them in the act? Towards imaging and tagging microbes on rock and mineral surfaces using SEM-ISH

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Microorganisms are constantly changing the world around them and can leave signatures in their environment, however techniques that 'catch them in the act' are limited, especially when you have limited information on 'them' and what actual 'act' they are performing. Fluorescent in situ hybridisation (ISH) has provided invaluable insights in medical and environmental research to identify microbes and how they interact, however fluorescent dyes are often unsuitable in geomicrobiology applications as many minerals will naturally fluoresce. Some researchers have used scanning electron microscopy (SEM), combined with ISH as the resolution is greater than with light microscopes. To achieve this standard 16s rRNA oligonucleotide probes were tagged with nanogold instead of fluorescent probes. We are developing techniques to fix cells on various rock and mineral surfaces alongside a robust method for SEM-ISH the identification of cells can be used along side standard SEM to describe the local substrate on which they are positioned. The techniques are compatible with use of standard ISH signal enhancement methods and the use of RNA probes for targeting mRNA, thus allowing us to locate and identify microorganisms carrying out particular 'acts'.

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Effects of temperature and Fe(II) concentrations on rates and extent of Fe(II) oxidation by nitrate-reducing bacteria in a marine sediment

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Bacteria capable of coupling nitrate reduction to Fe(II) oxidation (NRFeOx) were discovered 20 years ago and numerous efforts have been made to isolate NRFeOx bacteria. However, most of the described pure cultures can oxidize Fe(II) only under mixotrophic conditions and many of those that do not require an organic co-substrate for Fe(II) oxidation cannot be cultivated autotrophically over several generations. Therefore, the aim of this study was to use microcosms and stable-isotope probing (i) to identify active bacteria within a community under autotrophic NRFeOx conditions and (ii) to determine the effects of temperature and Fe(II) concentrations on active microbial populations. Samples from Aarhus Bay, Denmark, were pre-incubated (>70 days) with 2 mM nitrate to stimulate heterotrophic nitrate reduction and to deplete bioavailable organic carbon at either 20°C or 4°C. When nitrate reduction ceased and the nitrate concentration remained stable, the conditions were considered to be optimal for autotrophic growth. At this point either 2 mM or 500 µM Fe(II) and labeled/unlabeled bicarbonate were added. Nitrate reduction resumed in all setups and was coupled to Fe(II) oxidation. In the 2 mM setups at 20°C, 1.8 mM Fe(II) was oxidized at a rate of ~0.12 mM day^{-1} while in those pulsed with 500 µM, 2.4 mM Fe(II) was oxidized over the same period. Active bacteria in the microcosms were identified using nucleic acid-SIP. Our findings demonstrate the occurrence and activity of autotrophic NRFeOx bacteria in a marine sediment and show the implications of temperature and Fe(II) concentrations on active microbial populations.

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Persistence of microbial biosignatures with increasing metamorphic grade

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Little is known about the survivability of microbial biosignatures within rock substrates when subject to the extreme pressures (P) and temperatures (T) typically experienced during rock burial, yet our picture of early Earth biosphere and microbial evolution is based upon the preserved organic and geochemical remnants of such microorganisms. Using a piston-cylinder press we exposed a natural microbial biofilm community hosted within a thermal spring carbonate matrix to 6 different P-T conditions spanning 500 - 800 MPa and 200 - 550 °C (zeolite - amphibolite facies) to investigate the persistence of microbial organic material and associated geochemical biosignatures with increasing metamorphic grade. Experimental products were analysed with 2D Raman spectroscopy and FEG-SEM mapping to investigate the spatial and geochemical evolution between organic and inorganic phases. Microbial biofilms comprising filamentous sulfur oxidising bacteria, cyanobacteria, and diatoms were associated with elevated silica, which mineralised increasingly graphitised organic carbon as temperature and pressure increased. Discrete clusters of sulfur globules associated with the biofilms persisted across all metamorphic facies, becoming deformed at the highest PT conditions. Microbial organic matter underwent thermal maturation with increasing temperature. At the highest P-T conditions, graphitised organic carbon and sulfur globules were completely silicified and eventually preserved within a Quartz phase, while the carbonate recrystallised to form the surrounding matrix. These results demonstrate the value of experimental work within this field, providing insights into the micron-millimetre scale partitioning of organic and inorganic phases within natural materials relevant to early Earth environments.

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Methanogenic communities in Athabasca oil sands and their potential for bioenergy production

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Most of the oil in low temperature, non-uplifted reservoirs is biodegraded due to millions of years of microbial activity. We performed long-term microcosm experiments to investigate whether addition of inorganic nutrients and different electron acceptors could stimulate further biodegradation of already severely biodegraded Athabasca oil sands bitumen (Alberta, Canada) for the production of methane. We combined basal water and surface-mined bitumen in microcosms that were incubated for 3000 days with bitumen as the only organic substrate and with nitrate, sulfate, nitrate and sulfate, oxic headspace or no additional electron donors. Methanogenesis was observed in both replicates for three out of five treatments. Maximal rates were below 15 nmol methane/day/g oil sands. This is 10 to 1000x lower than other reports of methanogenesis from lighter crude oils. Methanogenic *Archaea* and several potential syntrophic bacterial partners were enriched, including sulfate-reducing bacteria. Interestingly, in microcosms that contained sulfate, methanogenesis took place in the presence of >20 mM sulfate that had not been removed. The organic composition of the bitumen before and after incubation was analysed by gas chromatography-mass spectrometry (GC-MS) and Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS). No significant degradation was observed for any compound or compound class, suggesting that degradation may have occurred at low rates and in an unspecific manner. These results demonstrate that microbial communities in Athabasca oil sands are capable of accessing a limited pool of organic carbon present in severely biodegraded oil as substrate for further biodegradation resulting in methanogenesis, but that rates are relatively low.

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Fungal transformation of cobalt-bearing ores: bioleaching and bioprecipitation

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Geoactive fungi such as *Aspergillus niger* play a significant role in bioweathering processes and element cycling. These organisms are able to secrete a range of metabolically produced organic acids, such as oxalic acid, into their microenvironment. This enables them to mediate mineral dissolution, leading to metal solubilization and also biomineralization in the form of secondary oxalate biominerals. In this investigation, such biotransformation processes by geoactive fungi were explored as a means of bioprocessing and biorecovery of cobalt – an element identified as being of key industrial and strategic importance. A range of Co-bearing laterite and pyrite ores were investigated, in addition to seafloor ferromanganese nodules, for their susceptibility to fungal biotransformations. Direct examination of fungal-colonized cobalt-containing substrates by light and scanning electron microscopy was used to reveal mineral transformations at the microscale. Bioleaching studies in liquid media revealed differences between the ore substrates, with bioleaching being more effective from laterites: in some cases >90% Co was released. The influence of mineral presence on the range of organic acids produced and the effectiveness of culture filtrates for bioleaching and/or mineral precipitation were also examined. The work contributes to understanding the factors that influence chemoorganotrophic cobalt bioleaching and biomineral precipitation at micro- and nanoscales, with the aim of optimising maximal cobalt biorecovery from solution.

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Identification of UDP-glucose 4-epimerase (*galE*) Gene Isolating from the Oral Metagenomics DNA Confers Resistance to Quaternary Ammonium Compounds (QACs).

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Use of antimicrobial compounds results in bacteria developing resistance. The human oral cavity is one environment exposed to these compounds through foods and oral hygiene products.

We have adopted a metagenomic approach to screen for resistance genes from both culturable and unculturable bacteria in the human oral cavity. An oral metagenomic library, constructed in Escherichia coli using pCC1BAC vector, was screened against several antimicrobials.

Out of 12,277 clones screened, one clone was found to have resistance against two commonly used antiseptics, cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC). Analysis of the plasmid from this clone showed that it contained a 17.1 kb insert, and the resistance was conferred by a UDP-4 glucose-epimerase (*galE*) gene homologous to one from *Veillonella parvula*. The product of *galE* is involved in LPS production. Analysis of the *E. coli* host showed the cell surface charge was more positive in the presence of *galE*, which could reduce the binding of these positively charges antiseptics to the bacteria.

This is the first time *galE* has been shown to be responsible for resistance against QACs.

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Cryogenic silicification of microorganisms from hydrothermal fluids

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The interaction of silica-supersaturated fluids with freezing temperatures is common on Earth, and has been throughout its history. When frozen, silica-rich hydrothermal fluids precipitate cryogenic opal-A (COA) silica particles which bear characteristic morphologies due to ice crystal templating. We investigated cryogenic silicification as a novel preservation pathway for microorganisms inhabiting terrestrial hydrothermal systems. Several phylogenetically and metabolically distinct strains relevant to geothermal environments were silicified to capture a range of preservation scenarios. These included thermophilic sulphate-reducing bacteria *Desulfovibrio indonesiensis* and *Thermodesulfovibrio islandicus*, the photoferrotroph *Rhodopseudomonas palustris* and the thermophilic methanogenic archaeon *Methanoculleus thermophilus*. Our observations show that microbial cells are co-partitioned with colloidal silica, resulting in encasement within cryogenic opal-A microparticles, as well as the formation of cell-shaped casts on the surface of COA particles. Natural COA particles from Strokkur, Iceland, were also found to contain similar biomorphic casts and voids. Organic biosignatures were detected via Fourier Transform infrared spectroscopy in association with cell casts on both natural and laboratory samples, demonstrating that chemical evidence for microbial activity can be recorded along with morphological biosignatures by COA sinters. Cryogenic co-partitioning of microbial cells with colloidal silica is therefore a previously undescribed mechanism by which microfossils can be generated in hydrothermal settings. This work has implications for the detection of life beyond Earth, such as on Mars or Enceladus, where both opal-A and evidence for hydrothermal activity have been detected, and for exploring microbial communities in terrestrial hydrothermal systems during times of sub-zero temperatures, both past and present.

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Cycles of evaporation and freshwater recharge facilitate increased microbial growth rates in an extremely concentrated magnesium sulfate brine

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Microbial growth in extremely concentrated brines is fundamentally limited by ionic composition. Water activity, pH, ionic strength and chaotropicity are composition-defined physicochemical parameters which have demonstrable effects on microbial proliferation. However, hypersaline waters are not static environments, and processes such as evaporation, freezing, freshwater recharge or the dissolution of other salts can significantly alter a brine's physicochemical profile. We investigated the growth response of a highly Mg^{2+} and SO_4^{2-} -tolerant *Marinococcus* isolate to simulated evaporation and dilution cycles in a Mg-SO_4-Na-Cl brine. Our results show that evaporative concentration can benefit microbial growth by altering the chemistry of the environment. Growth rates at later evaporitic stages remained the same despite large decreases in water activity. Moreover, these effects were retained upon freshwater recharge: dilutions of later evaporitic stages supported faster growth rates than earlier evaporitic stages at the same water activity. We found that additive salt-specific growth rates could not explain these trends; rather that bulk physicochemical changes, driven by ion ratio shifts during evaporation, were likely responsible. These results demonstrate the importance of ionic composition to defining microbial growth windows in brines, and how the effects of evaporation on microbial growth can only be understood in the context of the underlying geochemical changes. As brines with diverse chemistries exist on the Earth and on other bodies in the solar system, a new framework for understanding brine habitability is required, that accounts for the geochemical history of brines as well as the various stresses that ions impose on microbes.

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Isolation and identification of iron-reducing bacteria in East Anglia fresh water

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Iron is the fourth most abundant element in the Earth's, mostly found in nature as ferrous iron (Fe+2) and ferric iron (Fe+3). Some microorganisms can use ferric iron as an electron acceptor to accomplish anaerobic respiration. Water and sediment samples were taken from the river Yare (Norwich, United Kingdom) and cultured anaerobically in minimal media with ferric iron as the sole electron acceptor source with the aim of isolating new iron-reducing bacteria. Rates of bacterial growth and iron reduction were compared with the model organism for iron reduction, *Shewanella oneidensis*. Additionally, molecular and bioinformatic techniques were used in order to identify the proteins involved in the iron reducing pathway. The isolated strains corresponded to the *Acinetobacter* and *Citrobacter* genus. Both of these genus have species identified as opportunistic pathogens and have not been associated with iron reduction before. Contrary to *S. oneidensis*, these species did not express *c*-type cytochromes to facilitate extracellular iron reduction, nevertheless, they were able to grow while reducing different ferric iron chelates as sole electron acceptor when oxygen was not available for the cellular respiration. The identification of *Acinetobacter* and *Citrobacter* as iron reducing bacteria is not just important to expand the knowledge on how iron molecules are reduced and oxidized in fresh waters, but also gives a better understanding on how opportunistic pathogens can survive in the environment.

Emerson, D., Roden, E., and Twining, B.S. (2012) The microbial ferrous wheel: iron cycling in terrestrial, freshwater, and marine environments. Front. Microbiol. 3: 383.

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Impact of extreme pH changes on microbial communities and relevance to radioactive waste disposal

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Microbial activity in the vicinity of a geological disposal facility (GDF) for radioactive waste could have a number of negative (e.g. metal corrosion) or positive (retardation of radionuclides) impacts. A better understanding of microbiology in these environments is required to understand the likelihood of these impacts and to ensure the safe disposal of the waste. One of the key environmental changes around a GDF is the development of hyperalkaline conditions. Surface hyperalkaline sites have often been used to understand potential for microbial activity. However, little is known about how microbial communities from rocks with characteristics that might make them suitable for the location of a GDF respond when they are exposed to increasing pH conditions. To study this, eight microcosms containing crushed rock (Borrowdale Volcanic Group), the indigenous microbial community and artificial groundwater were set up under anaerobic conditions. Artificial groundwater was passed through the microcosms for eight months. Four microcosms maintained at pH8 were compared to four microcosms that were exposed to gradually increasing pH conditions up to a maximum of pH 12.5. All microcosms were then returned to pH 8. Rock samples were collected throughout the experiment for DNA analysis. Changes to dominant microbial species were monitored using denaturing gradient gel electrophoresis. On-going work is being carried out to link changes in water chemistry to changes in the abundance of different functional groups of microbes. The results are discussed in relation to their relevance to the safety case for geological disposal of radioactive waste.

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Scalable microbial production of functional nanoparticles with enhanced mobility and reactivity for remediation applications

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Biogenic nanomaterials show a potential for *in situ* remediation applications. However, their field applications are restricted due to constraints such as their cost effective production, poor mobility and low reactivity in natural sediments. Here we demonstrate for the first time how reactive nanoparticles of magnetite can be synthesized using the dissimilatory Fe(III)-reducing bacterium *Geobacter sulfurreducens* under ambient conditions in the laboratory, and their surface properties fine-tuned for enhanced mobility and reactivity, required for field applications.

Through a series of batch and columns studies, in combination with state of the art imaging and spectroscopy analyses, the optimal nanoparticle synthesis regimes were selected for a range of *in situ* applications aimed at the treatment of metal and organic contaminants. We demonstrate the applications of biogenic magnetite by using hexavalent chromium Cr (VI) and tetrachloroethylene (PCE) as model contaminants. In addition, the incorporation of nanoscale palladium (Pd) coatings to enhance the reactivity has also been investigated.

We further demonstrate how iron-rich natural sediments can be used as a starting material for the microbial production of reactive Fe (II)-bearing novel nanomaterials, offering cost effective, environmentally benign routes for the sustainable production of nano-scale remediation agents.

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The colonisation of early land masses – how does rock type define community assembly and habitability?

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The interactions between life and the geosphere are complex, yet poorly understood and modelled. They play a crucial role in biogeochemical cycling, defining the limits to life and ecosystem energy flow. We currently have a limited understanding of how the planetary crust selects for different microbial communities in the process of colonisation. We implemented an experiment to study the colonisation of two end-member igneous rock types representing the two major chemical compositions of the bulk of the Earth's crust, granite and gabbro, during the course of two years. The experiment was exposed to the atmosphere to allow for atmospheric inoculation. Microbial community composition is determined by 16S rDNA analysis each month, and abundance measured by cell and CFU counts. The study addresses the process of colonisation using the metacommunity concept, elucidating the role of species sorting and neutral assembly in defining the course of colonisation. After six months, the communities cluster according to rock type in a principal coordinate analysis, indicating that the geochemical environments selects for the resident community. However, the dispersal of the communities suggests a role for stochastic or neutral processes in community assembly. This experiment yields new insights into the factors that determine how new planetary crust becomes colonised and whether the bulk composition of a planet's surface has a significant role in determining the types of microbial communities that become established. Furthermore, by elucidating the link between microbial populations and substrate the experiment shows the extent of coupling between the geosphere and biosphere.

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Fungal biomineralization of copper carbonate nanoparticles

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The development of reliable and environmentally-friendly technologies for the synthesis and stabilization of nanomaterials has received extensive attention. Many microorganisms, including bacteria, yeasts and fungi, are able to produce inorganic materials in nanoscales intracellularly or extracellularly. The use of filamentous fungi for nanoparticle synthesis represents a promising scaffold since fungi are able to secrete large amounts of, e.g. organic acids, amino acids and enzymes that may be involved in nanomaterial synthesis: the effect of extracellular protein on nanoparticle crystallization has been pointed out in many previous studies. The aim of this study was to examine fungal biomineralization of copper carbonate nanoparticles. In particular, the importance or potential role of extracellular amino acids and proteins on mineral morphology was studied, which will provide further understanding of the mechanisms of nanoparticle formation. Here, precipitation of copper carbonate nanoparticles was investigated in urea-containing growth medium using the fungi *Neurospora crassa* and *Myrothecium gramineum*, which have been shown previously to be capable of metal carbonate precipitation through hydrolysis of urea. Abiotic synthesis of copper carbonate in the presence of selected amino acids and protein was also simulated. It is concluded that amino acids can regulate particle size and influence the mineral surfaces, and this may offer novel design principles for the control of nanoparticle synthesis.

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Imaging the impact of Fe(III) and As(V) reduction by Shewanella sp. ANA-3 using nanoscale Secondary Ion Mass Spectrometry (NanoSIMS)

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More than 100 million people are affected by the chronic exposure to arsenic (As) in groundwaters, particularly in Southeast Asia. As(V) sorbs to iron (Fe) minerals, and it can be desorbed by biotic and abiotic processes. Once desorbed, As(V) is reduced to As(III) in transformations catalysed by anaerobic Fe(III)- and As(V)-reducing bacteria, in a process that remains poorly understood. This study focuses on the microbial reduction of As(V) sorbed onto Fe(III)-oxyhydroxides by imaging the elemental distribution associated with this electron transfer mechanism using NanoSIMS.
Pure cultures of Shewanella sp. ANA-3 (Fe(III) and As(V) reducer) and a deletion mutant (non As(V)-reducer) were grown on silicon wafers, coated with an Fe(III)-oxyhydroxide with As(V) incorporated, and incubated with a medium containing 13C-labelled lactate, to identify active bacteria. The samples were preserved by chemical fixation, imaged in a SEM and analysed in a CAMECA NanoSIMS 50L ion microprobe. Data were analysed using ImageJ and the plugin OpenMIMS.
Active bacteria were separated spatially from the mineral, suggesting that direct contact with the electron acceptor was not required, likely due to the secretion of flavin redox mediators. The mineral coatings respired by active bacteria had lower NanoSIMS Fe counts in comparison with those in controls, suggesting the release of Fe, and confirmed by geochemical measurements. Imaging of the As relative to both Fe and the microbial biomass is ongoing, and will be presented in the context of improving our understanding of the nanoscale impact of microbial metabolism on arsenic in subsurface sediments.

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Metagenomic and Metabolomic analysis of Winogradsky columns to investigate the effect of molybdate supplementation.

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In the late 1800s, Sergei Winogradsky, who is considered the "Father of Soil Microbiology" developed a culture system now known as the Winogradsky Column. This culturing method creates a microcosm that is used to model complex soil environments. In this study, two, five-year-old Winogradsky columns were analysed for community composition and metabolic profile using Next Generation Sequencing and Mass Spectrometry. The columns were constructed with biomass from the University of Strathclyde Burn. One of the columns acted as a control whilst the other was supplemented with molybdate. It was hypothesised that the column containing molybdate should enrich for nitrate reducing bacteria as molybdate is used as an essential cofactor in this process. DNA was extracted from each column using the Mo Bio PowerSoil DNA Isolation kit and Whole Genome Shotgun metagenomics and 16S rRNA gene sequencing were carried out using an Ion Torrent PGM benchtop sequencer. Raw sequence data was analysed using Galaxy, Silva-NGS and MG-RAST. To extract the metabolites associated with each column, the organic matter was homogenised, incubated with Diaion HP-20 beads and centrifuged. The resulting pellet was extracted using ethyl acetate and the crude extract analysed using liquid chromatography tandem high-resolution mass spectrometry.

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Lithotrophic communities and biosignatures in geothermal environments in Iceland: an analogue for Mars

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To better understand the requirements for detecting ancient or extant microbial life on Mars, it is important to study analogues on Earth such as Iceland, which hosts a variety of hydrothermal and lacustrine habitats of relevance to Mars. Recognizing which microbial metabolisms would be realistic in early Martian environments can help us to understand which biosignatures may be detected, and which geological deposits to focus on.

On the Vatnajökull ice cap in Iceland, geothermally-sustained glacial lakes and ephemeral pools host lithotrophic microorganisms adapted to anoxic, oligotrophic environments, including organisms important in the global carbon, nitrogen, and sulfur cycles. This study will focus on microbial ecosystems at the Kverkfjoll volcano. We will examine this site to answer questions including: i) What microbial communities dominate different environments in Icelandic hydrothermal lake/pool systems? ii) What are the stable isotope biosignatures produced by these lithotrophic microbial communities? and iii) How do these geochemical biosignatures change under Mars-like conditions?

Sampling of microbial mats, sediment, and waters will be conducted during summer when there is interaction between melting ice and fumaroles/springs. We will conduct microbial community genomic studies to investigate the evolutionary relationships of the different phylotypes, their predominance in the different conditions, and their functional capability. These genomic analyses will be coupled with H, C, N and S isotope analyses to identify active biogeochemical processes, and establish which of these are recorded within the sedimentological record produced by the organisms in these environments.

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Bioavailability of PVC for microbial nitrate reduction at high pH

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Safe disposal of nuclear waste relies on a thorough understanding of the role that microorganisms play on the fate of key radionuclides over long time periods. A significant amount of nuclear waste is organic, for example PVC plastic, yet little is known about the bioavailability of such materials in the high pH environment of a cementitious geological disposal facility. To address this knowledge gap, we assessed whether PVC is available to a high pH-adapted microbial consortium to fuel nitrate reduction at pH10. Two forms of PVC were studied, a chemically pure powder and a flexible film containing plasticisers. Samples of both were irradiated using a ^{60}Co gamma source to a final dose of 1 MGy to determine the impact of radiation on bioavailability. Both PVC forms, irradiated and non-irradiated, were added as the sole carbon and electron donor source to nitrate-containing medium buffered to pH 10, and inoculated with sediment from an alkaline environment known to contain denitrifying bacteria.

PVC film was bioavailable for nitrate reduction at pH10, whether irradiated or not, though non-irradiated PVC film fuelled more nitrate reduction. Minor nitrate reduction was detected with irradiated PVC powder; non-irradiated powder did not fuel nitrate reduction. 16S rRNA sequencing indicated enrichment of common soil lineages not previously known to operate at high pH. The results suggest that plasticised PVC could fuel microbial metabolism under nuclear waste disposal conditions. Future work focuses on identifying the impact of these processes on radionuclide mobility and the safety cases for geological disposal facilities.

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Flavocytochrome at the microbe-mineral interface of *Shewanella oneidensis* under mineral respiring conditions

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The facultative anaerobe *Shewanella oneidensis* must respond to fluctuating oxygen and nutrient levels in their natural environment. This led to *Shewanella* species evolving systems to utilise a wide range of terminal electron acceptors, including solid minerals (e.g iron oxides) in the extracellular environment. This respiration is dependant on the bacteria transporting electrons from their inner membrane, across the periplasm and outer membrane to the terminal acceptors. The process is facilitated by the MtrCAB complex, a complex made up of two decaheme cytochromes (MtrA and MtrC) associated with a β-barrel porin (MtrB), spanning the outer membrane.
Flavin molecules have long been implicated in the process of moving electrons from the surface exposed MtrC to solid mineral terminal electron acceptors, the exact mechanism under debate. Recent advances in our understanding of the structure of MtrC has shown flavin molecules form tight associations upon reduction of the disulfide bond in domain 3 of the protein, suggesting reduction of the disulfide bond is necessary for electrons to move from cells, sustaining growth.
Alongside this discovery we have shown this disulfide bond acting as an oxygen sensitive switch. When the cysteine residues forming the disulfide bond are substituted cells struggle to grow under aerobic conditions, suggesting a toxicity to oxygen. Further investigation highlights increased levels of reactive oxygen species (ROS) such as hydrogen peroxide generated by oxygen interacting with the flavocytochrome as a potential cause for this. From this data we propose that the MtrC flavocytochrome to be the dominant mineral reductase under environmental conditions.

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Laying the foundations for a multi-omic perspective on microbial induced carbonate precipitation

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Despite the considerable potential for using microbially induced carbonate precipitation (MICP) in a variety of applications, such as bioconcrete or biogeoengineering, surprisingly little is known about the microbial physiology and genomics of key microbial agents used in ureolytic MICP, namely *Sporosarcina pasteurii* and *Sporosarcina ureae*. Motivated to redress these omissions we sought to develop a multi-omic insight to *Sporosarcina*.
First, we prepared a draft *S. pasteurii* genome at 120 fold coverage using Illumina HiSeq which showing a 3.2 Mbp (39% GC) genome with 3,111 genes. This indicated a substantial gene and genome reduction relative to publicly available *Sporosarcina* genomes yet the genome retained a full repertoire of essential genes, with abundant carbohydrate and amino acid metabolism systems. Importantly, the genome retained functional sporulation and urease related subsystems. Currently we are nanopore sequencing to improve *S. pasteurii* and *S. ureae* assembly.
Both species have been proposed for caprock fissure plugging in CO_2 storage reservoirs, but supercritical CO_2 ($scCO_2$) injection effects on *Sporosarcina* metabolism are not well known. As a prerequisite to high throughput, non-targeted LC-MS metabolome profiling in response to mesocosm $scCO_2$ injection we sought to develop a defined, minimal medium compatible with both authentic *Sporosarcina* growth and mass spectrometric profiling. On the basis of genome data, five minimal media were trialled relative to complex media and in conjunction with four trace element solutions. Our work sets the stage for an integrated genomic, transcriptomic and metabolomic perspective of how Sporosarcina survives $scCO_2$ injection to perform MICP to securely sequester CO_2.

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Role of *c*-type cytochromes and riboflavin on U(VI) and Np(V) reduction by *Shewanella oneidensis* MR-1

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Anaerobic and facultative anaerobic microorganisms, including *Shewanella oneidensis* MR-1, can use soluble radionuclide species such as U(VI), Tc(VII) and Np(V) as terminal electron acceptors and reduce them to insoluble U(IV), Tc(IV) and Np(IV) species. Thus, microbial metabolism can potentially contribute to the immobilisation of radionuclides in contaminated land or in a geological repository for radioactive waste. Although *c*-type cytochromes and flavins have been shown to be involved in dissimilatory metal reduction (e.g. for ferric iron) in *Shewanella* species, the mechanisms of microbial radionuclide reduction remain largely unexplored. Therefore, this study investigated the role of *c*-type cytochromes and flavins on U(VI) and Np(V) reduction, by comparing reduction rates of wild type *Shewanella oneidensis* MR-1 and two deletion mutant strains. The first mutant lacked the outer membrane *c*-type cytochromes MtrC, OmcA, MtrF, and the second one lacked the most important cytochromes beyond CymA. U(VI) was not reduced by the c type cytochrome lacking mutants, indicating that they are required for U(VI) reduction. Np(V) was however reduced by all the mutants, indicating a novel mechanism, independent of *c*-type cytochromes. For both U(VI) and Np(V), addition of riboflavin accelerated the reduction rates, highlighting the potential role of secreted flavins as extracellular electron transport mediators. The reduced solid phases were characterised by X-ray absorption spectroscopy, which confirmed reduction to insoluble U(IV) and Np(IV) bearing phases. Interestingly, the latter was identified as predominantly non-crystalline Np(IV), something that has never been observed before, and may have significant implications for the environmental fate of transuranic elements.

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Floc formation is a key survival strategy for microorganisms living under the hyper-alkaline conditions likely to occur in an intermediate level radioactive waste disposal site.

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The survival of microorganisms within a cementitious geological disposal facility for radioactive wastes is heavily dependent on their ability to survive the calcium dominated, hyper-alkaline conditions resulting from the dissolution of the cementitious materials. The present study shows that the formation of flocs, composed of a complex mixture of extracellular polymeric substance (EPS), provides protection against alkaline pH values up to pH 13.0. The flocs were dominated by Alishewanella and Dietzia sp, producing a mannose rich carbohydrate fraction incorporating extracellular DNA, resulting in Ca2+ sequestration. This EPS provided a ~10µm thick layer around a central aggregation of cells facilitating an internal floc pH which was 0.6 to 0.8 pH units lower that the external pH values of pH 11.0 and 11.5. The floc structure allowed bacteria growth at pH 11.0 and 11.5, survival at pH 12.0, and a reduced floc survival (<2 weeks) at pH 13.0.This study is the first to demonstrate that flocs are able to maintain a lower internal pH in response to hyperalkaline conditions expected to occur within a cementitious, geological disposal facility for radioactive wastes and indicate that floc communities within such a facility would be capable of survival up to a pH of 12.0.

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Cornish mining heritage: a microbial ecology study

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Mining in the south west of England began in the early Bronze Age approximately 2150 BC and the last tin mine in Cornwall closed in 1998. Historically extensive tin and copper mining has occurred in Crnwall and Devon, as well as arsenic, lead, silver, zinc and other metals. No metalliferous mines are active. However, mine waste or mine derived heaps are still abundant in the environment and are characterized by an increasing level of weathering. Cornish mine environmental waste represents a unique natural laboratory to study acidophile, lithotrophic microbial communities evolving towards a more heterotrophic microbial composition and their relationship to the geochemical characteristics of the mineral they are hosted by. Twelve sites in Cornwall and western Devon, have been chosen for this study and for each site two sub sites and a control were considered. Geochemical features (i.e. soil total metals, pore water metals content, organic carbon) and microbial ecology were studied. Prokaryotic 16s rDNA V4 hypervariable region was analyzed through Illumina sequence analysis. Results indicate a consistent diversity core present in the most of samples and species richness not highly variable across the sites and controls, a major range of differences among the samples is given by the distribution of OTUs and diverse distributions are found in the sites defined by more "extreme" geochemical features. We are currently working on the data analysis and on "bioprospecting" and isolating effort in order to name the many uncultured OTUs present in the samples, especially deriving from subsurface sites.

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The geomicrobiology of trace elements in soils from tropical ecosystems in the Santa Elena Peninsula, Costa Rica

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Costa Rica has a broad set of ecosystems favoured by its location in the neotropic, the influence of the oceans and its geology. Despite its small size, it is one of the most biologically diverse countries in the world with 4% of the total biodiversity on Earth. In the north-western part of the country is the Santa Elena Peninsula, a section of the Guanacaste Conservancy Area declared as World Heritage by UNESCO in 1999 due to its unique ecological characteristics. In terms of its geology, the peninsula comprises an ultramafic complex composed mainly by peridotites, dunites and gabbros, and is tightly related with the ecosystems present in its landscape. The natural weathering of ultramafic rocks has led to iron and magnesium dominated soils, with potentially toxic levels of other metals including nickel, cobalt and chromium.
The aim of this research is to study the biogeochemical cycling of cobalt and other trace elements in the soils of the Santa Elena Peninsula. The first objective of the investigation is to describe the nature of the cobalt in the soil samples, understanding its geological origin and its effect on the soil microbial communities present. A second objective will seek to analyse the relationship between anaerobic and aerobic processes during the biogeochemical cycling of cobalt and other heavy metals. Initial results from a multidisciplinary approach, including mineralogical characterization techniques as XRD and XRF, geochemical profiling and next generation DNA sequencing will be presented.

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The Alteromonadales, Rhodobacterales and Flavobacterales; master recyclers in coastal waters

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Phytoplankton blooms occur annually in coastal waters and have a functionally diverse bloom-associated bacterial population. While phytoplankton blooms contain a seasonally variable and diverse community of dominant taxa, a few heterotrophic bacterial lineages, the orders Alteromonadales, Rhodobacterales and Flavobacterales, dominate bacterial communities during and after blooms in coastal waters. These orders are potentially important recyclers of both particulate and dissolved organic matter produced by phytoplankton blooms. Our work has previously correlated abundance of both Rhodobacterales and Flavobacteriales with a decline in a specific phytoplankton produced fraction of particulate organic carbon, transparent exopolymer particles (TEP). Our current work has directly linked Alteromonadales, Rhodobacterales to degradation of TEP using enrichment mesocosms and high-throughput sequencing of 16S rRNA transcripts combined with RNA stable isotope probing (SIP). We have also used metatranscriptomics to attempt to identify some of the potential carbohydrate active enzymes involved in the breakdown of TEP. Further experiments looking at the degradation of dissolved DNA (dDNA) using enrichment mesocosms and RNA-SIP have linked these groups to the degradation of a significant component of the dissolved organic matter pool. These results add further evidence to the growing wealth of literature that these orders of bacteria, along with several others, are well adapted to life amongst the phytoplankton and are able to utilise a diverse range of components from phytoplankton-derived organic matter pools. These orders of bacteria are ecologically important parts of the microbial loop and are involved in nutrient recycling in multiple biogeochemical cycles within surface coastal waters.

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Heavy metal inducible antimicrobial activity of Streptomyces spp. isolated from the Leadhills and Wanlochead lead mines.

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There is a real and urgent need for new antibiotics to combat the rise of antibiotic resistance. There are also an increasing number of reports that correlate resistance to heavy metals with antibiotic resistance. It has also been shown that triggering the expression of cryptic gene clusters in Streptomyces might yield novel antibiotics. Here, we isolated Streptomyces strains from sediments contaminated with heavy metals from a former industrial site in Scotland and these strains were assayed for heavy metal dependent antimicrobial activity. We have used a combination genomics and transcriptomics to investigate these novel strains, providing a phylogenetic context and molecular evidence towards the discovery of the gene clusters responsible for antibiotic biosynthesis.

Our findings highlight the potential of using heavy metals for activation of silent secondary metabolite gene clusters in Streptomyces isolated from extreme environments for natural product discovery. These findings are supported by the fact that these compounds are produced only in presence of sub-inhibitory concentrations of heavy metals but not in the absence of metal induction.

Whole genome sequencing has enabled us to investigate the taxonomy and biosynthetic capacity of the strains whilst transcriptomic and metabolomic analyses are allowing us to investigate the changes that occur under metal-inducing conditions.

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The Enrichment of Alkali Tolerant Methanogenic Communities from Anthropogenic Lime Contaminated Sites

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The survival of methanogens at alkaline pH values is an important consideration for the disposal of intermediate level radioactive wastes. Within a cementitious geological disposal concept, carbon substrates (including acetic acid) will be generated from the alkaline hydrolysis of cellulosic materials. In addition, hydrogen may be generated via corrosion and microbial processes. The utilisation and generation of these gases by methanogens is likely to have an impact on the evolution of a geological disposal facility by influencing gas pressures.

Within this study, sub-surface sediments from lime-contaminated environments were used as inoculum for alkaline (pH 10.0) methanogenic enrichment cultures. Enrichments were performed through the addition of either H_2/CO_2 or sodium acetate to promote hydrogenotrophic and acetoclastic methanogenesis. The enriched sediments were able to generate methane from hydrogen and carbon dioxide within 4-8 weeks. No methane was generated from sodium acetate fed systems.

Microbial communities were identified via Illumina MiSeq sequencing. Archaea were below the limits of detection within the soil fraction, but were the dominant taxa following enrichment. Methane generation from all sites can be attributed to the taxonomic family Methanomicrobiaceae, with sequencing reads showing homology to the genus Methanoculleus. Taxa of the family Methanomassiliicoccus were present in six of the enrichment cultures, representing 2% - 11% of the communities. The family Methanobacteriaceae was only present in one of the enrichments.

In summary, hydrogenotrophic methanogenesis is common within alkaline environments. Furthermore, Methanoculleus, a hydrogenotrophic methanogen, appears to be the most abundant, alkali tolerant methanogen within the habitats sampled in this study.

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Bistable Flagellar Gene Expression across Serovars of the pathogen Salmonella enterica

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The flagellum is a remarkable organelle embedded into the bacterial cell wall. It consist of three regions a basal body, a hook and a filament. More than 60 proteins contribute together to assemble the flagellum. There are three classes of promoter that drive expression of flagellar genes. In the upper part of hierarchy is flhDC. FlhDC is very crucial protein complex which regulates class II promoter activity. The pathogenic species Salmonella enterica exhibits a robust motility phenotype with the vast majority of Salmonella serovars being motile. In this study, we investigated the magnitude and timing of flagellar gene expression across S. enterica serovars.

We will demonstrate that the timing of flagellar gene expression is consistent across the species but the magnitude of flagellar gene expression varies significantly. The S. enterica flagellar system is bistable, producing a heterogeneous population of motile cells. To ask if the magnitude variability was a result of bistability we will present data that assesses flagellar gene expression in the population and the consequences of FlhDC protein stability. Our data suggests that population heterogeneity plays a role in the adaptation of S. enterica serovars to their hosts with respect to motility.

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Three enzymes and a substrate; regulation of flux through a "non-canonical" metabolic branchpoint.

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Pseudomonas aeruginosa is a common opportunistic pathogen. Recent work indicates that in many infection scenarios, P. aeruginosa exhibits an exquisite predilection for metabolising fatty acids to yield acetyl-CoA. In most higher organisms, acetyl-CoA cannot be used for biomass production because the two carbon atoms which enter the TCA cycle are lost as CO_2. However, many bacteria are able to bypass these oxidative decarboxylation steps, allowing them to conserve carbon for gluconeogenesis. They do this by using the "glyoxylate shunt". Here, isocitrate is cleaved by isocitrate lyase (ICL) to yield succinate and glyoxylate (which, in a subsequent reaction, is combined with a further acetyl-CoA unit to yield the gluconeogenic precursor, malate). However, ICL has to compete with the TCA cycle enzyme, isocitrate dehydrogenase (ICD), for the available isocitrate, and it is the outcome of this "metabolic tussle" which dictates the flux of carbon through the glyoxylate shunt. In E. coli, ICD is inactivated by AceK-dependent phosphorylation, allowing flux through the glyoxylate shunt. However, P. aeruginosa is "wired up" differently because it employs not one, but two highly-expressed isocitrate dehydrogenases (ICD and IDH). I found that only one of these (the E. coli-like ICD) is regulated by AceK-mediated phosphorylation. The other, IDH, is allosterically regulated, as is the isocitrate lyase. I have solved the x-ray crystal structures of ICD, IDH and ICL, and characterized their regulatory properties and kinetics. I am using these data to build up a picture of how flux is likely to be regulated at this "non-canonical" metabolic branchpoint.

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Structural analysis of the E. coli ammonium transporter AmtB

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Ammonium transport across biological membranes is a fundamental process in all living organisms and is mediated by the Amt proteins. The functional importance of Amt protein is highlighted by their central role in nitrogen metabolism in bacteria, fungi and plants, and their signaling function in the induction of filamentous growth, a dimorphic change often associated with virulence in pathogenic fungi. Despite the importance of these proteins there is still an incomplete understanding of the links between their structure and function.

We have undertaking a novel multidisciplinary approach, using Small angle neutron scattering, Small angle X-ray scattering and Cryo-electron microscopy to fully understand the functions of this remarkable family of transporters. Our specific aim is to interrogate their dynamics and more precisely characterise the molecular basis of the conformational rearrangement that occurs during the substrate transport cycle. We have recently obtained very exciting low resolution structures, hence opening the possibility to apply our approaches to visualise conformational change of the protein upon substrate binding.

There is a considerable interest to investigate the conformational changes associated with the function of membrane transporter. The current technical difficulties in obtaining such information require new approaches such as developing and implementing new biophysical techniques in this biological field. In the future integrated approaches, which combine biochemical, biophysical, computational analysis and low resolution structures, are likely to provide a standard framework for the mechanistic understanding of membrane protein structure/function relationships.

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2C or not 2C? Regulation of carbon flux through the glyoxylate shunt in the opportunistic pathogen, *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa (PA) is the major pathogen associated with chronic and ultimately fatal airway infections in patients with cystic fibrosis (CF). The glyoxylate shunt is an anaplerotic pathway that allows micro-organisms to grow on acetate or fatty acids as a sole carbon source – a feat that most higher-organisms are incapable of. For this reason, the junction between the glyoxylate shunt and the TCA cycle is widely considered to be one of the most important metabolic branchpoints in microbial metabolism.The glyoxylate shunt is a widely-accepted target for the development of antimicrobial compounds. However, the enzymology of the branchpoint between the TCA cycle and glyoxylate shunt in PA differs substantially from that in well-characterized organisms such as E. coli, and little is known about how flux through the shunt is regulated in PA. We have utilised a combination of transcriptomic, proteomic and metabolomic approaches to investigate this metabolic branchpoint in considerable detail. Comparative RNA-Seq analysis and metabolomics of PA grown on glycerol or acetate as sole carbon sources identified relevant pathways and metabolites which might impinge upon branchpoint regulation. Factors controlling enzyme expression levels are being investigated by using DNA-affinity purification; this has identified several previously uncharacterised transcription factors which bind to the upstream region of the genes encoding various branchpoint enzymes. Finally, the formation of multi-enzyme mega-complexes is being investigated using formaldehyde cross-linking combined with mass spectrometry. Collectively, these approaches are allowing us to develop a multi-scale model accounting for the regulation of flux through the glyoxylate shunt in PA.

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Evolutionary Dynamics of a bacterial weapon: *Photorhabdus* Virulence Cassettes

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Photorhabdus bacteria harbour a vast array of potent toxins. Acute lethality against the insect host via use of these toxins is vital to symbiosis with *Heterorhabditid* nematodes. The *Photorhabdus Virulence Cassettes* are one such mechanism. Each strain harbours 5-6 distinct 27kb "PVC" operons, of ~18 genes. These encode a 'nanosyringe'-like complex, that translocates genetically coupled effectors to target cell interiors. PVC operons share gross structural similarity, resembling bacterial tailocins, but the coupled effectors are extremely diverse. PVC operons have the hallmarks of horizontal acquisition, and diversity among different homologs suggests they are prone to recombination. Bioinformatic analysis indicates that many PVC operons are linked to remnants of insertion elements and in some cases have the configuration of composite transposons. Via collection of basic sequence statistics and gene-versus-species phylogenetic congruency analysis we infer the degree to which the PVCs are recombining and how they may be evolving. We also infer the degree to which paralogy within an operon is driving ORFs to fixation/removal. This provides insight into the effect of gene copy on the assembly stoichiometry of the PVCs, and the redundancy within the operons.

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Determining the mechanism of BAM-assisted OMP folding

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Outer membrane proteins (OMPs) mediate the survival and pathogenicity of Gram negative bacteria. The biogenesis of these proteins however, presents problems as they must be transported to, inserted and folded correctly in the outer membrane in the absence of ATP. The β-barrel assembly machinery (BAM) complex is a ~203 kDa complex of five proteins (BamA-E) that enables the membrane insertion and folding of substrate OMPs on a physiological timescale. Despite available crystal structures the mechanism of this vital protein complex remains poorly understood.
We have used structural and biochemical tools to probe the nature of BAM-assisted OMP folding. We present the first cryo-electron microscopy structure of the complex, at a resolution of 4.9 Å. This reveals the intact BAM complex with BamA in a laterally-open conformation, between the first (β1) and last (β16) strands of the barrel. This structure reveals conformational changes relative to existing crystal structures and displays interactions between subunits and the detergent micelle, suggesting modes of communication between BAM and the lipid bilayer.
In addition, we have examined the hypothesis that BamA may assist substrate folding via lateral gating between the β1 and β16 strands. Our functional assays provide the first in vitro evidence of this, demonstrating that in a reconstituted system, inhibiting the lateral gating of BamA diminishes the ability of BAM to assist substrate folding. Combined, the data presented advance our mechanistic understanding of BamA function in isolation, and as part of the intact BAM complex.

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The *Bacillus subtilis* Signal Recognition Particle (SRP): Known unknowns

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Introduction: The SRP is a ribonucleoprotein complex that is essential for protein secretion. Much of what is currently understood about bacterial SRPs is derived from *Escherichia* coli. However, the structures of SRP in Gram-positive and Gram-negative bacteria are different, the former having two domains ("S" and "Alu") the latter just one ("S" domain).

Methods: While Gram-positive and Gram-negative SRPs play similar roles, their modes of action may are likely to be distinct, particular with respect to translational arrest. To analyse the structure and activity of the *Bacillus subtilis* SRP, we have used combination of electromobility shift assays (EMSA) and Microscale Thermophoresis (MST). Full-length SRP RNA (scRNA), and Alu and S domains were synthesised and DIG-labelled *in vitro*, using T7 RNA polymerase. Putative protein components were purified by immobilised metal affinity chromatography (IMAC) and analysed with SDS-PAGE.

Results: The data have revealed novel insights into the composition of *B. subtilis* SRP. We have evidence that two proteins, YlxM and TufA, form a complex with the S domain of the scRNA. We have also shown that the previously reported binding of Hbs to the Alu domain is likely to be an experimental artefact, associated with this pleiotropic protein's role in DNA packaging.

Conclusions: We have uncovered novel features of the *B. subtilis* SRP. However, we still need to better understand the functional roles of the scRNA and protein components in SRP-mediated protein secretion.

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The activation of RNase E by binding of structured RNAs to an allosteric site

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Escherichia coli endoribonuclease E is a major regulator of gene expression and homologues are found in many bacteria and in some plant plastids. RNase E cleaves RNA by the simultaneous recognition of multiple unpaired regions or an unpaired region(s) in combination with a 5'-monophosphorylated end. Indeed, it is believed that the cleavage of RNA by RNase E is limited by the affinity of interaction. Here we show for the first time that the 5' leader and 3' trailer sequences within tRNA precursors are both required to permit a strong interaction of the substrate with the RNA-binding channels of NTH-RNase E. We also provide evidence that the binding of these unpaired regions is not always sufficient to mediate cleavage, and that an adjacent tRNA unit acts as a cis-regulatory RNA element that enhances catalysis. RNase E activation may be achieved by the interaction of these supporting tRNA units with an allosteric binding site found within the NTH. We propose that the cooperative interaction of two or more unpaired regions of RNA is required to increase the affinity of the substrate for RNase E, and that neighbouring stem loop structures are required to initiate cleavage and hence increase the selectivity of RNase E. This would explain why several cleavages by RNase E are found adjacent to complex stem loop structures.

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Following colicin translocation to the *Escherichia coli* cytoplasm one molecule at a time

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Gram-negative bacteria often release toxic bacteriocins into their immediate environment as a means of competing with closely related microorganisms. Colicins are potent bacteriocins expressed and released by E. coli that bind to outer membrane protein receptors with high affinity and, following contact with proteins in the periplasm and inner membrane, translocate a cytotoxic domain into the cell by a poorly understood mechanism. Cell-death occurs in minutes through depolarization of the cell, hydrolysis of peptidoglycan precursors or digestion of nucleic acids in the cytoplasm. The dogma in colicin research is that a single molecule is sufficient to kill a cell, although this has yet to be formerly demonstrated. The aim of this research was to develop fluorescence-based microscopy tools to visualise colicin entry into E. coli as a test of this hypothesis and to probe the mechanism of translocation through the cell envelope.
We have developed fluorescence widefield and total internal reflection fluorescence (TIRF) microscopy methods for investigating the import of single colicin E9 molecules in E. coli. We demonstrate that ColE9 fluorescently-labelled in its C-terminal endonuclease domain retains catalytic activity and cytotoxicity. Using this fluorescently-labelled ColE9 we have for the first time visualised the translocation of individual colicin molecules to the cytoplasm of E. coli, tracked the diffusion of these single molecules in vivo and demonstrated that import is dependent on the proton motive force. This research will describe current progress in exploiting these newly developed tools in understanding bacteriocin translocation in bacteria.

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Species wide analysis of *Escherichia coli* identifies a negative regulatory input that controls *flhDC* transcription

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The flagellar system of *E. coli* is organized into a transcriptional hierarchy dependent on the regulation of the flagellar master regulator *flhDC*. Previous studies of MG1655 suggested that *flhDC* transcription is dependent upon IS element upstream of the *flhDC* promoter. Screening uropathogenic *E. coli* clinical isolates with hypervariable motility phenotypes found only 1 of 50 to contain a *flhDC* linked IS insertion. Up to 10 % of *E. coli* genomes assessed exhibited similar IS insertions. This suggested IS insertion may not be a dominant factor in dictating motility in *E. coli* isolates.Bioinformatic analysis of the *flhDC* promoter showed that it reflected the phylogenetic order of *E. coli*. Further analysis between *flhD* and the upstream gene *yecG* four sub-regions: a) untranslated *flhDC* leader sequence; b) the *flhDC* promoter; c) an upstream regulatory region for P_{flhDC} and d) a SCAR region showing sequence variability. The SCAR region was named as it is the known location of previously reported IS element insertion sites.We have asked what impact the intragenic region has upon motility, avoiding IS element insertion. We will present an analysis of *flhDC* promoter activity and promoter switching experiments from various *E. coli* strains. Deletion analysis identified the SCAR region to negatively impact motility. We propose that the dependence on IS insertion in certain *E. coli* strains dysregulates the negative regulation of *flhDC* transcription. Our data suggests even though the *yecG-flhDC* region is prone to IS element attack, other factors dictate the regulation of *flhDC* transcription across *E. coli* as a species.

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The power of DNA rings: unravelling the segregation mechanism of the low copy number plasmid pB171 in Escherichia coli

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The stable inheritance of bacterial low copy number plasmids is mediated by dedicated active segregation systems. Escherichia coli enteropathogenic strain B171 specifies virulence factors that cause diarrhea and are encoded by the low copy number plasmid pB171. The plasmid contains two partition cassettes. The system under investigation is the par2 module, which consists of three elements: two genes, parA encoding an ATPase and parB encoding a DNA-binding protein, and two partition sites, parC1 and parC2, which harbour direct repeats and recognized by the ParB protein.To determine the minimal partition site necessary for pB171 segregation, the DNA repeats were progressively deleted from the parC1 and parC2 sites and plasmid partition assays were carried out. The interaction of ParB with the partition site was investigated by performing electrophoretic mobility shift assays (EMSA) and by using microscale thermophoresis (MST).Plasmid partition assays have indicated that the parC1 site is dispensable for pB171 stable maintenance, as no obvious plasmid loss was observed when most of the repeats were deleted. This finding argues for a gene regulatory rather than partition role for the repeats in parC1. In contrast, deletion of the parC2 repeats abrogates plasmid retention completely, highlighting that this partition site is crucial for pB171 stability. EMSAs and MST experiments have shown that ParB binds parC1 site with high affinity and the kinetics of the interaction is currently being investigated. Mutagenesis and structural studies are also presently under way to achieve a fuller understanding of the mechanism of action of the ParB protein.

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Investigations into the Type VII secretion system of *Staphylococcus aureus* and the role of secreted effectors

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The secretion of protein effectors is an essential process that allows bacteria to readily adapt to changing and challenging environments. A number of virulence factors are known to be exported by complex secretion systems and can modulate host immune responses. One such system is the Mycobacterial Type VII secretion system (T7SS), components of which have been discovered in a variety of bacteria including the notorious human pathogen *Staphylococcus aureus*. Recent research from the Palmer lab has led to the characterisation of a secreted anti-bacterial toxin EsaD from *S. aureus*, which possesses a nuclease domain and targets the DNA of other *S. aureus* strains. Two further proteins were found to be involved in the biogenesis of this toxin; a chaperone EsaE, and an anti-toxin EsaG. Interestingly, analysis of *S. aureus* genomes from different clonal complexes has highlighted the diversity of the *ess* operon, which encodes the components and predicted substrates of the T7SS. Current work is focussed on using genetic and biochemical techniques to further understand the differences between these operons and determine the biological activities of any secreted effector proteins. In this study, particular emphasis is placed on the *S. aureus* strain EMRSA15 with gene deletions generated and characterised, and predicted toxins investigated.

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A genetic screen reveals evidence for posttranslational control of protein translocation in bacteria

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Sodium azide inhibits bacterial growth by inhibiting SecA, an ATPase required for the translocation of proteins across the cytoplasmic membrane. Using a method known as TraDIS (transposon-directed insertion site sequencing), we screened a high-density transposon-insertion library for mutations that increase sensitivity to azide. Amongst these were insertions in genes encoding components of the Sec machinery and cell envelope biogenesis machineries were more sensitive to azide, which we confirmed using single-gene deletion mutants. In addition, functional clustering of the most depleted insertion mutants in TraDIS and gene expression profiling of azide-treated cells suggest that azide disrupts metal-ion homeostasis. Depletion of metal ions in the growth media increased sensitivity to azide, and supplementation with iron, but not zinc, decreased sensitivity to azide. In contrast to other sec genes, insertions in the secA gene, which truncate the non-essential C-terminal extension of the protein, become enriched during growth in the presence of azide. Cells expressing higher levels of SecA were more sensitive to azide. *In vivo* pull-downs, ^{1}H-NMR and EPR experiments suggest that Fe^{2+} is the natural ligand of the MeBD and that azide disrupts the structure of the MeBD by interacting with Fe^{2+}. Finally, azide-resistant *secA* mutants are impaired in their ability to recover when iron uptake was inhibited using lipocalin-2, suggesting that SecA play a role in the physiological response to perturbations in iron homeostasis. Our results suggest that the strong and apparently specific inhibition of SecA by azide is likely the result of a confluence of a diverse number of mechanisms.

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Lateral opening and membrane interactions in the intact β-barrel assembly machinery captured by cryo-EM

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The β-barrel assembly machinery (BAM) is a ~203 kDa complex of five proteins (BamA-E) which is essential for viability in *E. coli*. BAM promotes the folding and insertion of β-barrel proteins into the outer membrane via a poorly understood mechanism. The complex is surface-located, essential, and highly conserved making it an attractive target for the development of novel antibacterials against Gram-negative pathogens. Several current models suggest that BAM functions through a 'lateral gating' motion of two strands in the membrane spanning β-barrel of BamA. Here we present a cryo-EM structure of the detergent solubilized BamABCDE complex, at 4.9 Å resolution and additional cryo-EM structures of the complex in other membrane mimetic systems. The detergent structure is in a laterally open conformation showing that gating is independent of BamB binding. We describe structural variation in different membrane mimetic systems, and interactions between BamA, B, D, and E and the detergent micelle/membrane mimetics that suggest communication between BAM and the lipid bilayer. Finally, using an enhanced reconstitution protocol and functional assays, we show that for the outer membrane protein OmpT, efficient folding *in vitro* requires lateral gating in BAM.

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Characterisation of a novel membrane-targeting toxin delivered by the bacterial Type VI secretion system

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The Type VI secretion system (T6SS) is widely distributed amongst Gram-negative species. T6SSs are encoded by large and variable gene clusters, however within these loci is possible to recognize fourteen genes that are strongly conserved. These genes encode fourteen highly conserved "core" components, named TssA-M and PAAR, which form a large, envelope spanning machinery, enabling delivery of toxins directly into eukaryotic or prokaryotic target cells.
To date, it has been shown that many anti-bacterial T6SS-dependent toxins can be categorized into different families: peptidoglycan amidase and glycosidase enzymes, phospholipases, probable membrane pore forming proteins and nucleases, while other candidate toxins still have an unknown function. These anti-bacterial toxins are encoded adjacent to specific cognate immunity proteins. The presence of immunity proteins provides protection against self-killing and attack from sister cells. Previous work has identified one T6SS in *S. marcescens* Db10, which targets and efficiently kills other bacterial cells and plays a role in anti-bacterial competition. While some of the toxins have been characterized and their function has been determined, little is known about the others. The aim of this project is to characterize one of these newly identified toxins, including validation of its role as an antibacterial toxin and its association with a cognate immunity protein together with initial investigation into its molecular function. We confirmed that inhibition of the activity of the toxin in sister cells depends on direct interaction with a cognate immunity protein. Additionally, we have generated new insight into its mode of action against target cells.

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Targeting the Type Three Secretion Syringe to Combat Infection

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Anti-virulence (AV) compounds are a promising alternative to traditional antibiotics for fighting bacterial infections. AV compounds act specifically on bacterial virulence factors instead of simply killing the bacteria or inhibiting the growth cycle, resulting in less evolutionary pressure for the development of resistant strains. The Type Three Secretion System (T3SS) is a well-studied and attractive AV target, given that it is widespread in more than 25 species of Gram-negative bacteria, including enterohaemorrhagic *E. coli* (EHEC), and since it is essential for host colonisation by many pathogens. In this work, we designed, synthesized and tested a new series of compounds that block the functionality of the T3SS of EHEC. A detailed evaluation of our AV compounds highlighted that two of the new compounds effectively blocked secretion in a dose dependent manner. Affinity chromatography experiments identified the primary target of the compounds as the T3SS needle pore protein EspD, which is essential for effector protein translocation into host cells. These data were supported by mechanistic studies that determined the coiled-coil domain 1 of EspD as the compound-binding site, thereby preventing correct assembly of the T3SS complex on the cell surface. Additionally, we found the compounds to exhibit dual-functionality by also down-regulating transcription of the entire chromosomal locus encoding the T3SS, further demonstrating their desirability and effectiveness. The ease of synthesis, solubility and selectivity of these compounds support them as an excellent baseline for future AV drug design to combat EHEC virulence.

Macromolecular Machines

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Shedding light on the molecular mechanism of genome segment counting in segmented RNA viruses

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A major challenge in understanding of assembly of segmented RNA viruses, including pathogenic rotaviruses, is the combinatorial problem of genome segment assortment, during which distinct single-stranded (ss)RNA copies of segments are selected from a cellular pool of ssRNAs. Such process involves complex RNA-RNA and protein-RNA interactions, which often remain obscured due to non-specific binding and aggregation at concentrations approaching in vivo assembly conditions. In order to understand the molecular basis of the 'molecular counting' of multiple genomic RNA segments, we employ a single-molecule fluorescence cross-correlation spectroscopy (FCCS) combined with pulsed interleaved excitation to interrogate inter-segmental RNA-RNA interactions in rotaviruses. We show that such interactions require binding of the viral non-structural ssRNA-binding protein NSP2. We identified sites of these interactions by introducing an RNA-RNA SELEX (Systematic Evolution of Ligands by Exponential enrichment) methodology and we demonstrate that rotavirus ssRNAs undergo conformational rearrangements upon binding of NSP2. Our findings elucidate the molecular basis underlying inter-segmental interactions in rotaviruses, paving the way for future mechanistic studies of assembly of other segmented RNA viruses, as well as non-coding RNAs interacting with their targets.

Annual Conference 2017
EICC, Edinburgh
3-6 April

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Phylogenetic and pathogenic characterisation of *Mauginiella scaettae* as the causal agent of date palm (*Phoenix dactylifera* L.) inflorescences rot

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Unavailable

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Root Hairs are a Determinant of the Rhizosphere microbiota

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The bacterial communities thriving at the root soil interface, termed the rhizosphere microbiota, can perform beneficial roles for the plant host including facilitating nutrient uptake and aiding pathogen defence. To obtain novel insights in to plant host traits that shape these microbial communities I characterised the microbiota populating the rhizosphere of two pairs of near-isogenic barley (*Hordeum vulgare*) genotypes, contrasting for root hair characteristics grown in two different Scottish agricultural soils. Rhizosphere specimens were subjected to high resolution 16S rRNA gene profiling using an Illumina MiSeq protocol. Sequencing data were subjected to ecological and multivariate statistical analysis to define to what extent root hairs shape the rhizosphere microbiota. Remarkably, mutant plants displayed a reduced-complexity microbial community compared to unplanted soil controls and their wild type counterparts. The host root hair phenotype consistently explained up to 18% of the variation in the bacterial community composition of the rhizosphere samples. Intriguingly, deeper analysis of the sequencing profiles showed that this effect is linked to the differential recruitment of a taxonomically small range of bacteria, showing a bias for members of the orders Actinomycetales, Burkholderiales, Rhizobiales, Sphingomonadales and Xanthomonadales. To ultimately determine how these taxa are causally linked to given plant traits, I have developed recolonization assays to quantify the impact of synthetic bacterial communities on rhizosphere formation and functioning.

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Characterization of endophytic Streptomyces and its application for blast disease control

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Three actinomycete isolates, RRS-8, RRL-8 and RRB-2 were screened for fungicidal activity against Pyricularia oryzae, the causal agent of the rice blast disease. All isolates was identified using 16S rRNA gene analysis as Streptomyces. The result of growth inhibition of P. oryzae using dual culture method on solid medium revealed that all isolates could inhibit the mycelial growth of P. oryzae. The fermentation broths of these isolates grown in yeast extract-malt extract medium at 30 ^{o}C for 14 days on rotary shaker (180 r.p.m.) were freeze dried into the solid powder. These solid powders were used for testing the anti-fungal activity. The result showed that the solid powder at 0.4% (w/v) of isolate RRS-8 exhibited the best concentration for suppressing the mycelial growth of P. oryzae. Applying the 0.4% solution of solid powder of RRS-8 in 0.025 % tween 20 in water on the leaf of rice grown in the greenhouse at 12 h pre-inoculate with P. oryzae (2.53×106 spores/ml) exhibited 97.9% disease reduction of rice blast while the 0.4% solution application after pathogen inoculation revealed even lower rates of disease reduction.

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Discovery of pathogenic *Labyrinthula* spp. (Labyrinthulomycota) in Scottish saltmarshes producing rapid blight symptoms in turfgrass: a risk to saltmarsh or golf-courses?

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Background: Rapid blight disease of turfgrass destroys golf-courses with saline soil and/or irrigation water (>0.5 dS/m). Pathogenic protists, *Labyrinthula terrestris*, are the only Labyrinthulomycete known to cause disease outside marine environments therefore, a survey of saltmarshes, where a corresponding mix of salinity and hosts occur, was conducted to determine their environmental origin.

Methods: In March-July 2016, healthy and dead grasses were sampled from 17 saltmarshes throughout Scotland. After surface sterilisation, plant fragments were plated on semi-selective medium for *Labyrinthula* spp. Both SSU and LSU-ITS sequences were obtained for all successfully isolated strains. Pathogenicity to susceptible turfgrass species *Poa trivialis* was assessed for 6 different isolates, representing each genetic group, as previously described[1].

Results: Twenty-seven *Labyrinthula* spp. were successfully isolated from 12 locations. Both SSU and LSU-ITS resolved these isolates into 4 phylogenetic species groups. Pathogenicity trials revealed two as non-pathogenic under the experimental conditions. Two were highly pathogenic to *P. trivialis*. One isolate was 100% identical to the rapid blight strain previously isolated from Scotland; 'Laby31'. This is the first record of rapid blight pathogens out-with golf-courses. The other pathogenic strain represents a putative new species, with unique genotype and morphology.

Conclusions: *Labyrinthula* spp. appear common in saltmarshes, with 4 different genotypes and potentially novel species being isolated using culture methods alone. The occurrence of both known and new pathogenic *Labyrinthula* spp. in saltmarsh grasses has important implications for golf-course disease management and saltmarsh conservation.

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A hairy situation: can plants recognise and respond to *E. coli* fimbriae?

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Escherichia coli O157:H7 are well known food-borne pathogens that can spend some of their life cycle in the environment by colonising plant hosts. This ability poses a food safety threat when the bacteria colonise crop plants that are minimally processed prior to consumption. Previous work in our lab has shown that *E. coli* O157:H7 adhere to plant tissues via flagella, *E. coli* Common Pilus (ECP) and F9 fimbriae. As its name suggests, ECP is conserved amongst the *E. coli* species, with homologues present in *Klebsiella* species. The F9 fimbriae are encoded on an operon which is prevalent in human pathogenic strains of *E. coli* such as O157:H7 and uropathogenic E. coli (UPEC).

In light of this, we wanted to investigate whether the plant recognises the presence of these organelles to initiate a defence response. The transcriptomic response of *Arabidopsis thaliana* Col-0 leaves to infiltrated flagella, ECP and F9 fimbriae was tested using microarrays. Flagella were included to act as a positive control as the MAMP response to flagellin is well documented in the literature. No response has been reported, as yet, to bacterial fimbriae even though these organelles are involved in initial attachment and biofilm formation. Genes involved in the specific response to fimbriae are identified through comparison with those induced by flagella and from other published datasets. These results expand the repertoire of MAMPs recognised by plants to control microbe colonisation.

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Fungicidal activity of aromatic medicinal plants against Myrothecium roridum Tode associated with Myrothecium leaf spot disease of Momordica charantia L. (bitter gourd)

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Introduction: Myrothecium roridum Tode emerges as a potential threat for the bitter gourd crop in Pakistan since 2007. Aromatic medicinal plants were used for its management under greenhouse and field conditions.
Methods: A total of seven aromatic medicinal plants [garlic (Allium sativum), onion (Allium cepa), ginger (Zingiber officinale), green chilli (Capsicum frutescens), capsicum (Capsicum annuum), turmeric (Curcuma longa) and arvi (Colocasia esculenta)] were intercropped with the bitter gourd for the In vivo evaluation of fungicidal activity. Ten days old Myrothecium roridum cultures, maintained on potato bitter gourd dextrose agar medium were used as spray (@2x103spores/ml) for the artificial inoculation on four weeks old seedlings.
Results: Among the test plants, garlic, onion and green chilli exhibit a remarkable antifungal potential. Garlic lowers the disease incidence up to 63% under greenhouse and 58% in field experiments followed by the chilies (52% under greenhouse and 49% in field) and onion (41% under greenhouse and 39% in field). Ginger (27% under greenhouse and 22% in field) and capsicum (24% under greenhouse and 17% in field) shows a moderate antifungal potential while turmeric (11% under greenhouse and 5 % in field) and arvi (9% in pots and 8% in field) were least effective.
Conclusion: Intercropping of garlic, green chilli and onion can significantly reduce the Myrothecium leaf spot disease incidence in farmer fields. Further studies to evaluate the active chemical constituents responsible for their antifungal potential may help in the development of effective fungicides.

Keywords: Myrothecium roridum Tode, aromatic medicinal plants, fungicidal activity, in vivo.

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"A Search for Alternative Microbiological Controls of Plant Pathogens."

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Three plant pathogenic genera*, Fusarium*, *Phytophthora* and *Hymenoscyphus* cause significant losses to the cut flower industry and woodlands of Northern Ireland. Various soil treatments have proven largely unsuccessful in controlling *Fusarium*. Hence a study was made to source alternative microbial means of control of these genera, using soils taken from infected sites.

Bacteria and fungi were isolated from infected soils by plating of soil dilutions onto Potato Dextrose Agar (PDA). Those organisms showing antimicrobial activity were single colony purified, and their inhibitory properties further studied using dual-control assays on PDA. Agar plugs of pathogens were also laid onto lawns of dilution series of inhibitors, to determine numbers of inhibitors necessary to control pathogen growth. Inhibitory activity was compared with a serial dilution series of a garlic extract and selected commercial biostimulants of plant growth, to assess these for pathogen inhibition potential.

These assays isolated 4 bacterial species that were found to be inhibitory to *Fusarium*, *Phytophthora* and *Hymenoscyphus.* Concentrations of > 3% of the garlic extract and plant biostimulants proved totally inhibitory to the growth of all three pathogens. The tree pathogens proved to be more sensitive to microbiocides than *Fusarium*, which displayed a range of species-specific inhibition.

Based on these results, *Paenibacillus* and *Bacillus* isolates were chosen for further trialling as microbiocides, as suspensions for direct application to both soils and foliage, in both pot and field trials, to assess their potential primarily as prophylactics in controlling these and other plant pathogens.

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Microbial and circadian manipulations as alternative natural methods for suppression of cut flower wilt pathogen *Fusarium oxysporum*

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Poly tunnel roofed field grown cut flower scented stock, *Matthiola incana*, under standard lighting and temperature regimes encounters *Fusarium* wilt disease symptoms. Commercial fungicides are expensive and impose health risks. Natural and biological alternatives are desirable but not fully explored. In the light of the background knowledge upon light and temperature compensated circadian clock that regulates fungal growth and development, we evaluated the prospects for developing practical means of suppression of *Fusarium* wilt when the soil surface was covered with polythene sheets and the plant plugs treated with drip and/or drench applications of our local wilt suppressant isolates *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Paenibacillus polymyxa* isolated from our previous study or commercial microbiocide, Serenade Max (*Bacillus subtilis* QST713). The dip in thermal patterns between surface and 5-cm deep soil core obtained over an 8-week period in covered soils to those of uncovered soils influenced *Fusarium* population distribution and pathogen colonisation in the emerging host root zone in the early phases of disease development. Scanning electron microscopic examination revealed the presence of bacteria attached to the *Fusarium* hyphae. Further isolation on culture plate and 16S rRNA PCR analyses indicated that these ectosymbiont bacteria were predominantly *Pseudomonas flourescens, Stenotrophomonas maltophila* and such predisposition of hyphal surface to native bacteria have well established implications to fungal virulence regulation towards the host. The results support the generic prospects of manipulations such as induced darkness, lowering of temperature and utilising native microbiological sources as alternative fungicides for *Fusarium* wilt disease control in field environment.

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Generation of disease resistant crops expressing species-specific protein antibiotics

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Bacterial species belonging to several genera are immensely destructive plant pathogens resulting in tens of billions of pounds worth of crop damage per annum. A big contributor to this is the rod-shaped gamma proteobacteria *Pseudomonas syringae*. *P. syringae* consists of over 50 known pathovars, all of which cause different diseases such as bacterial speck, spot and blight disease on tomato, beans, tobacco and a large number of agronomically important crops. Numerous chemical interventions aimed to attenuate *P. syringae* outbreaks have ultimately failed. Innovative approaches are required to obtain a robust immunity phenotype against *P. syringae*. A potential novel solution to control *P. syringae* outbreaks is by generating disease resistant plants expressing protein-specific antibiotics produced by bacteria called bacteriocins. Bacteriocins are produced by all major bacterial lineages and their primary function is to kill closely related bacterial competitors to allow the producing strains to establish dominance within a niche. Consequently, we hypothesize that bacteriocins can be used as an effective strategy of controlling bacterial plant pathogens. We present *in vitro* and *in vivo* evidence demonstrating that the bacteriocin Putidacin L1 isolated from *P. syringae* is a promising candidate to transform into crops.

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Differences in internalisation and growth of *Escherichia coli* O157:H7 within the apoplast of edible plants, spinach and lettuce, compared with the model species *Nicotiana benthamiana*

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Internalisation of food-borne bacteria into edible parts of fresh produce plants represents a serious health risk. The internalisation of verocytotoxigenic *E. coli* O157:H7 isolate Sakai into the leaves and roots of spinach (*Spinacia oleracea*) and lettuce (*Lactuca sativa*) has been demonstrated and compared to the model species *Nicotiana benthamiana*. Here we demonstrate the marked differences in the fate of these endophytic *E. coli* O157:H7 Sakai.

In the fresh produce species infiltrated with a low inoculum dose (~10^2 CFU), bacterial growth was restricted but viable cells persisted over 20 days, whereas there was > 400-fold (~2.5 Log_{10}) increase in growth in *N. benthamiana*. This was supported with confocal fluorescence microscopy where only isolated bacteria were visible in spinach, lettuce or tomato (*Solanum lycopersicum)*, but large colonies formed adjacent to epidermal cells and mesophyll cells or close to vascular bundles of *N. benthamiana*. These colonies contained components of a biofilm maxtrix, including curli expression and elicitation, extracellular DNA and a limited presence of cellulose.

The data show that internalisation is a relevant issue in crop production and that crop species and tissue need to be considered as food safety risk parameters.

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Rapid resistance evolution can shape the antibacterial activity of plant growth promoting Pseudomonas bacteria

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Soil-borne pathogens are significant threats to food security, one economically important bacterial pathogen being *Ralstonia solanacearum* which causes bacterial wilt epidemics in over 200 plant species from 50 different families. One way to control disease levels could be to use plant growth promoting bacteria (PGPB) such as *Pseudomonas*. Antibiosis is one important mechanism to suppress pathogenicity against plant hosts. However, it is unclear if pathogenic bacteria can evolve resistance to antimicrobials produced by *Pseudomonas*. We used an experimental evolution approach to investigate if pathogenic *Ralstonia* bacteria (7 UK isolates) were able to evolve resistance to 8 different plant growth promoting *Pseudomonas* bacterial strains in various concentrations of antimicrobials. We found increasing antimicrobial concentration increases *Ralstonia* growth inhibition. However, this effect becomes weaker through time, which suggests that pathogens are evolving more resistance. Specifically, pathogens seem to be able to evolve resistance more easily at lower antimicrobial concentrations. Interestingly, some pathogen strains could evolve resistance faster than the others. Similarly, different *Pseudomonas* strains had different biocontrol efficacy. Together, our results suggest that rapid resistance evolution could weaken the efficacy of PGPB. However, this outcome could depend on the specific pathogen and PGPB strains involved. Considering PGPB bacterial effect on an evolutionary timescale could potentially explain often observed inconsistent biocontrol results in the field.

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The identification and characterization of novel haemolysin genes from *Clostridium difficile*

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Clostridium difficile is a Gram positive, spore forming, motile bacilli that is the leading cause of antibiotic associated diarrhoea and pseudomembranous colitis. *C. difficile* infection imposes a significant burden on the healthcare system with 250,000 cases per year resulting in 14,000 deaths and excess medical costs of $1 billion annually. The main virulence factor of this bacterium is the production of toxins, mainly A and B. Other factors have been reported to be involved in pathogenicity but the picture is not complete. Up until today, there are no reports of *C. difficile* expressing β-haemolysis when grown on blood agar plates. But when 2% glucose is added to the media, *C. difficile* shows haemolysis. To identify genes that could express haemolysis, a genomic library of *C. difficile* strain 630 was constructed in *E. coli*. Three clones were picked based on their large zone of β-haemolysis and the inserts were sequenced. The genes conferring a haemolytic activity were identified by subcloning of each gene separately. Six genes expressed a haemolytic phenotype. To our knowledge this is the first report of *C. difficile* conferring haemolytic activity. These genes might contribute to pathogenesis of *C. difficile* and further work is required to prove it.

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ADAPTIVE LABORATORYEVOLUTION OF AN *Escherichia coli*STRAIN LACKING PHOSPHOTRANSFERASE SYSTEM: REPRODUCIBILITY OF THEGENOTYPE-PHENOTYPE RELATIONSHIP

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Background. ALE was employed to improve the growth rate on glucose (glc) as sole carbon source, of an *E. coli* strain lacking phosphotransferase system (PTS-). It was demonstrated that a 10 Kb chromosomal deletion that include *galR* and *rppH* genes, caused a 250% recovery of growth of the PB12 evolved strain, isolated from this experiment.

To determine how reproducible the genotype-phenotype relationship is, and to study from a metabolic perspective the evolutive landscape, ALE was repeated.

Methods. Three independent ALE experiments from the same PTS- strain were performed. The occurrence of spontaneous mutants was monitored, and those with higher growth rate were characterized and submitted to WGS. Singles and doubles inactivations of mutated genes were performed in the PTS- strain, to evaluate its role in the adaptive process.

Results. Selected strains showed growth increases among 172% and 253%. The lost fragment identified in PB12 strain was found in one of the triplicates, however the fitness of this strain is lower compared to others that not lost it. Mutations in *galR* gene were observed in all experiments, however this gene does not have a significant effect on the growth rate increase. New beneficial mutations in genes as *rng*, contribute greatly to the recovery fast growth on glc were found.

Conclusion. The glc+ phenotype can be reached following different evolutionary paths. Despite do not find reproducibility in the genotype, we discover common mutations, which indicates the action of natural selection as result of a selective pressure.

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Persistence of *Enterococcus faecium* under extreme nutrient stress

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Enterococcus faecium is a Gram-positive commensal of the human gut. In the last few decades, it has acquired resistance to several antibiotics and has become an important cause of nosocomial infections. Although infections caused by *E. faecium* are not particularly severe, they can be difficult to treat due to its antibiotic resistance. Notably, *E. faecium* can survive outside the human host for extended periods of time, which increases its ability to spread throughout the hospital and increase the chance of outbreaks.
Our goal is to uncover the mechanisms used by *E. faecium* to survive outside the human host. Using high-throughput transposon sequencing (Tn-seq), we determined which genes are important for the survival of *E. faecium* E745 (a vancomycin-resistant, clinical isolate) under nutrient-limiting conditions at ambient temperatures. A transposon library of *E. faecium* E745 was incubated in phosphate buffered saline at 20oC for up to 7 days after which transposon insertion sites were determined by sequencing. Genes contributing to survival under nutrient stress covered a wide variety of functional classes, including general stress response, metabolism, transcription factors and membrane homeostasis. To validate genes found in the screening assay we are developing a novel genome editing strategy, using CRISPR-Cas9, to efficiently generate deletion mutants. As a proof-of-principle, we have deleted the gene encoding the large subunit of β-galactosidase in *E. faecium* E745.

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New Insights Into Bacterial Chromosome Origin Structure And Function

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Faithful genome duplication is essential for cell viability in all domains of life. The master bacterial replication initiation protein DnaA binds to the origin of replication (oriC) by recognizing DnaA-box elements (5'-TTATCCACA-3'). Interestingly, bacterial replication origins are diverse; they contain distinct numbers and orientations of DnaA-boxes. Although bacterial replication origins have been investigated for many years, the core set of DnaA-boxes required at any bacterial origin is unknown.
Using a unique strain of Bacillus subtilis that allows mutagenesis of the endogenous origin, DnaA-boxes were individually and then sequentially inactivated to abolish DnaA binding activity. This analysis revealed a distinct subset of DnaA-boxes necessary and sufficient for origin activity. Further analysis suggests that the spacing and orientation of the DnaA-boxes is critical for origin function. This work provides new insight into bacterial replication origin structure and function.

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Evolution of colistin resistance in clinical Klebsiella pneumoniae isolates and its impact on fitness and virulence characteristics

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Klebsiella pneumoniae has emerged as an important cause of multi-drug resistant nosocomial infections, leading to a resurgence in use of the last-resort antibiotic colistin. Colistin destabilizes the outer membrane of Gram-negative bacteria through electrostatic interactions with lipid A moieties of lipopolysaccharide.
To study development of colistin resistance in K. pneumoniae, we used four clinical, colistin-susceptible strains and evolved these in vitro to high-level resistance by culturing in the presence of increasing concentrations of colistin. SNPs and indels in isogenic colistin-susceptible/resistant strain pairs were determined through whole genome sequencing. Growth fitness, lipid A composition, susceptibility to human serum and human antimicrobial peptide LL-37, and membrane permeability were determined.
Susceptible K. pneumoniae readily evolved high-level resistance to colistin (MIC ≥128 µg/ml in Lysogeny Broth). Resistance was associated with mutations in genes encoding the PhoPQ two-component system, the LPS-assembly protein LptD, and the regulator of LPS biosynthesis YciM. None of the strains had identical mutations. In the resistant isolates, lipid A modifications were identified. One K. pneumoniae strain was negatively affected in its fitness by resistance. Permeability of the outer and inner membrane was significantly decreased upon exposure to LL-37 and colistin in the resistant isolates versus the susceptible parental strains. Three strains exhibited decreased susceptibility towards LL-37. Susceptibility to complement-mediated killing in serum increased for two strains, while the other strains were unaffected.
These results highlight the relative ease by which K. pneumoniae can evolve colistin resistance, through multiple evolutionary trajectories, with variable effects on virulence characteristics, and without appreciable fitness costs.

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Transfer of Plasmids from *Escherichia coli* to *Clostridium difficile* is Sensitive to DNase

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Background

Horizontal gene transfer (HGT) between bacterial cells is an important mediator of bacterial evolution and adaption to various environments. In the clinical setting it is a major player in the spread of antibiotic resistance. Three mechanisms of HGT in bacteria are known: conjugation, transduction and transformation. HGT is also used to transfer genetic constructs made in organisms that have relatively advanced genetic technologies, such as *Escherichia coli,* to organisms that are not as amenable to genetic manipulation. Commonly plasmid RK2 is used to mobilise plasmids having a compatible origin of transfer *oriT*. This system is used for genetically modifying the important human pathogen *Clostridium difficile.* In this work we demonstrated *oriT* is not required for transfer between *E. coli* and *C. difficile* and that transfer is abolished in the presence of DNase indicating that a possible cell-to-cell transformation-like mechanism is responsible for transfer.

Objectives

To investigate the mechanisms of transfer of plasmids between *E. coli* and *C. difficile*

Methods

E. coli donor strain CA434 (HB101 carrying the IncPß conjugative plasmid, R702) was transformed with pMTL9301 or pMTL9301Δ*oriT*. *E. coli* donors containing the plasmids was mixed with *C. difficile* CD37 anaerobically.

Conclusions

Deletion of *oriT* lowered the transfer frequency of pMTL9301 but did not stop it showing that pMTL9301 can be transferred by a mechanism different from conjugation. In support of this pMTL9301Δ*oriT* could not transfer to *C. difficile* in the presence of DNase. We hypothesize that an unknown DNA uptake system, possibly a cell-to-cell transformation-like mechanism is involved.

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Characterisation of the Putative Terminator Located Upstream of the Tn916 Conjugative Transposon.

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Tn916 is a clinically important conjugative transposon as it is a vector for the dissemination of antibiotic resistance among a broad range of bacteria. We identified a putative rho-independent terminator, 170 bp from the end of Tn916 and upstream of the conjugation genes. The predicted termination efficiency was 31%. To verify this experimentally, the terminator was cloned in between the tet(M) promoter and a gusA reporter gene in a pHCMCO5 shuttle vector. By measuring the β-glucuronidase activity of Bacillus subtilis containing the constructs, we demonstrated the level of enzyme activity decreased by 90% when comparing the construct containing the terminator to the construct containing the promoter only, confirming termination activity. We hypothesized that the terminator is preventing the transcription of the conjugation genes when Tn916 is integrated in the host genome. To test this, we cloned the terminator region into pHCMCO5 plus either the flanking DNA (representing the linear, integrated form) or the ligated ends of Tn916 (representing the excised and circularized form). The enzyme activity observed is twofold higher in the construct representing the circularized form compared to the construct representing the linear, integrated form. This data supports our hypothesis that the terminator efficiency is modulated upon excision and circularization of Tn916, which is the exact time when Tn916 would require expression of its conjugation genes.

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Control on the complex *Pseudomonas aeruginosa* ribonucleotide reduction network by the AlgZR two-component system

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DNA synthesis is one of the central processes of life, required for any living cell. The Ribonucleotide Reductase (RNR) family include the only enzymes able to catalyse the reduction of the ribonucleotides to deoxyribonucleotides, the building blocks of the DNA. This finely regulated family comprises three classes of enzymes (class I, class II and class III); although eukaryotic cells codify only class I, bacteria can codify any combination of classes. By its essentiality and its strong differences with its eukaryotic counterpart, bacterial ribonucleotide reductases are considered a promising field for the designing of new antimicrobial therapies.

In the opportunistic pathogen *Pseudomonas aeruginosa,* famous for its metabolic versatility and adaptability, we find all three classes codified in the same genome. It is believed that under the different situations this bacterium encounters, in planktonic growth and in biofilms, under free growth and under infections conditions, all three RNR classes are controlled by complicated regulation systems and play different roles.

In this work, we demonstrate the fine regulation of class I and class II RNR in *P. aeruginosa* by the AlgZR system, a two-component system responsible for the regulation of alginate biosynthesis, type IV pili production and twitching motility, as well as many other phenotypes. We investigated how the AlgZR system helps to finely tune up the expression of RNR classes to adapt to different growing conditions and environmental situations, intertwining for the first time these two regulation networks.

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Significance of the chromosomal positions of the genes encoding nucleoid-associated protein IHF in *Salmonella enterica* serovar Typhimurium.

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Heterodimeric bacterial nucleoid-associated protein IHF binds DNA specifically introducing U-turns. This property lies at the basis of its roles in shaping the nucleoid and assisting DNA recombination, replication and the regulation of gene expression. The IHF regulon includes genes involved in adaptation to stationary phase together with genes responsible for motility, chemotaxis and virulence. IHF is expressed to the maximum level during the transition from the exponential to the stationary growth phase. In *Salmonella* its subunits are encoded by *ihfA* and *ihfB* genes that are differentially regulated and moreover, located in different chromosomal positions - in the Ter and the Right macrodomains respectively. In this project the open reading frames (ORFs) of *ihfA* and *ihfB* in Salmonella are reciprocally exchanged while keeping the native 5' and 3' regulatory regions in place. The physiological properties of the resulting strain are compared with those of the wild type. Swapping the ORFs means that the same regulatory input will lead to the production of different mRNA output. The impact of this rewiring will be assessed at the levels of IHF expression, neighbouring genes expression, and global cellular transcription patterns. The study will deepen our understanding of the significance of chromosomal gene positions which is of interest in the context of synthetic biology. Here, this knowledge can be used to create artificial gene regulatory networks and/or to manipulate existing ones, expanding the available approaches to engineer cells for practical applications.

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Development of CRISPR-Cas9 in *Bacillus* sp. for the construction of industrially relevant strains

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The discovery of Clustered regularly interspersed short palindromic repeats (CRISPR) and the corresponding CRISPR associated (Cas) proteins, have recently revolutionised all areas of biological research. While the technology was developed from bacterial systems, uptake for use in bacterial biotechnology has been relatively slow. This is perhaps in part due to well characterised methods being in place, thus lowering the incentive for tool development, and the lack of clarity regarding the intellectual property surrounding CRISPR-Cas9.

Recently, CRISPR-Cas9 genome editing was exemplified in the generally regarded as safe (GRAS), Gram positive industrial workhorse, Bacillus subtilis. However, this technology has yet to be exemplified in other Bacillus sp. which are also industrially relevant. This technology has the potential to increase the success rate of genomic modifications in poorly transformable bacteria.

Here we describe the progress made in developing CRISPR-Cas9 based tools for the development of Bacillus sp. strains with commercial interest using a flexible system to edit the expression of industrially relevant targets.

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Understanding the Regulation and Mechanisms Involved in Multiple Antibiotic Resistance in Enterotoxigenic *E.coli*

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Antibiotic resistance in bacteria is a serious problem worldwide. Multiple antibiotic resistance in bacteria can be driven by the transcriptional regulators in the AraC-XylS family. The *Escherichia coli* '*mar*' regulon is considered a paradigm for such systems. The *mar* locus consists of 3 genes; *marR*, *marA*, and *marB*. A transcriptional activator encoded by *marA* enhances drug resistance by binding to "marbox" sequences at target promoters. The best characterised MarA targets encode the AcrAB-TolC drug efflux pump. We have identified 64 new MarA targets by ChIP-Seq analysis in the pathogen Enterotoxigenic *E.coli* (ETEC). Analysis of MarA targets has revealed novel mechanisms of resistance to quinolones and tetracyclines. This research provides new insights into the understanding of antibiotic resistance on a molecular level.

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An evaluation of purified *Salmonella* Typhi protein antigens for the serological diagnosis of acute typhoid fever

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The diagnosis of typhoid fever is a persistent challenge. Aiming to develop a specific serological diagnostic for typhoid we measured antibody against *Salmonella* Typhi (*S*. Typhi) protein antigens and the Vi polysaccharide in a cohort of febrile patients in Bangladesh. Twelve *S*. Typhi antigens previously shown to have diagnostic potential were cloned, expressed and purified. IgM against the 12 purified antigens and the *S*. Typhi Vi polysaccharide was measured by direct ELISA in plasma from patients with confirmed typhoid fever (n=32; 16 cases confirmed by blood culture, 13 cases confirmed by PCR and 3 cases confirmed by both blood culture and PCR), other confirmed infections (n=17) and 40 healthy controls. IgM responses were examined to identify individual antigens, or antigen combinations, that resulted in the best biomarkers for acute typhoid. ELISAs using most specific antigens were additionally performed on a collection of plasma from 243 patients with undiagnosed febrile disease. Acute IgM responses to the majority of *S*. Typhi protein antigens strongly correlated with each other (rho>0.8), but not against Vi (rho<0.6). Typhoid patients exhibited higher IgM measurements against 11/12 protein antigens and Vi than healthy controls and those with other infections. Individually, Vi, PilL (pilin component) and CdtB (typhoid toxin component) exhibited the greatest specificity and sensitivity (0.82; 0.68, 0.82; 0.62 and 0.82; 0.62, respectively) for identifying typhoid patients. However, specificity and sensitivity was improved when Vi was combined with a protein antigen, generating a specificity and sensitivity >0.85 and 0.80, respectively.

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Use of whole genome sequencing for the public health surveillance of *Shigella flexneri*.

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Shigella species are the most common cause of bacterial dysentery (bloody diarrhoea) worldwide. In England and Wales between 2007 and 2012, *S. flexneri* was the second most commonly isolated species (37.7%) compared to *S. sonnei* (49.9%), *S. boydii* (7.6%) and *S. dysenteriae* (4.34%). *S. flexneri* can be phenotypically serotyped using antisera raised to type specific somatic antigens and group factor antigens, and genotypically serotyped by the detection of O-antigen synthesis or modification genes. Recently, whole genome sequencing (WGS) has been implemented at Public Health England for the surveillance of bacterial gastrointestinal pathogens. The aim of this study was to evaluate the use of WGS for public health surveillance of *S. flexneri*. In total 333 *S. flexneri* isolates were sequenced in a perspective analysis with Illumina (Hiseq 2500) sequencers from the beginning of August 2015 to end of January 2016. In parallel with sequencing the 333 isolates were also analysed via traditional serotyping methodology. Of the isolates sequenced (333), 323 isolates (97%) had matching sequence and traditional serotyping results. The 10 (3%) mismatches results were caused by single nucleotide polymorphism in the O antigen synthesis or modification genes or novel combinations of these genes. In addition, seven outbreaks were identified, linked to an outbreaks the community, with a food outlet and that associated with transmission between men who have sex with men. WGS provides a robust approach for serotyping *S. flexneri* with the added benefit of providing epidemiological and phylogenetic data for use in outbreak detection and investigation.

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The roles of CRP and σ^{70} in regulating pathogenicity of *Vibrio cholerae*

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Vibrio cholerae is a Gram negative bacterium which is the etiological agent of Cholera, a disease affecting 4.3 million people each year, resulting in up to 140,000 deaths (WHO, 2015). The main symptoms of Cholera include profuse watery diarrhoea and vomiting, leading to coma and death in serious cases. The El Tor biotype of *Vibrio cholerae* is globally predominant, and is responsible for the 7th ongoing pandemic of Cholera. The success of this biotype to cause disease is thought to be, at least in part, due to the ability of the organism to survive well in two different environments; i) aquatic environments in coastal areas and estuaries, where *V. cholerae* can colonise the surfaces of shellfish, and ii) the human intestinal tract.

In this work, we have attempted to elucidate the regulatory mechanisms behind the switch between these two environments. We have used ChIP-seq (chromatin immunoprecipitation coupled with deep sequencing) to investigate genome wide binding of the cyclic AMP receptor protein (CRP) and the RNA polymerase σ^{70} subunit across both chromosomes of the prototypical *Vibrio cholerae* El Tor strain N16961. We show that CRP is involved in targeting genes involved in switching from an aquatic to a human intestinal environment. Genes targeted by CRP include those involved in biofilm formation on shellfish exoskeletons, chitin metabolism, and colonisation of the human intestine. In addition, we demonstrate a possible role for multiple adjacent CRP binding sites located within an intergenic region between two divergent genes, each encoding a haemolysin.

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***Pseudomonas aeruginosa* strain prevalence, adaptation and diversification during chronic lung infections of UK non-Cystic Fibrosis bronchiectasis patients**

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Although the evolution and adaptation of *Pseudomonas aeruginosa* (PA) populations during chronic lung infections of cystic fibrosis (CF) patients has been well studied, much less is known with regards to non-CF bronchiectasis (BE). Using isolates from 93 BE patients known to have chronic PA infection (enrolled in a trial of nebulised antibiotic therapy), whole genome sequencing was used to (i) assess the diversity of PA strains causing infections, (ii) assess the prevalence of multi-lineage (strain) infections, (iii) look for evidence of cross-infection or common source acquisition and (iv) characterise both adaptive mutations and within-population heterogeneity. Using the Illumina platform, 189 isolates underwent whole genome sequencing. Genomic data were used for typing by multilocus sequence type (MLST) and core genome SNP phylogeny, and to analyse genetic variations. The BE isolates were widely distributed within the global PA population structure. Of 23 patients from whose samples multiple isolates were examined, there were 6 examples of multi-lineage infections. SNP phylogeny revealed examples of more than one patient attending the same centre being infected with a common lineage. During infections of BE patients, PA populations adapt by accumulating loss of function mutations, leading to switches in phenotypes such as mode of iron acquisition and the production of biofilm-associated polysaccharides. As in CF, PA populations in chronic infection of BE patients exhibit considerable within-patient heterogeneity.

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The abundances of phosphatidylserine synthase mRNA and protein are uncorrelated in *Vibrio parahaemolyticus*

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Unavailable

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Revolutionising Drug Discovery Using Billions of Years of Evolution

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Natural small molecules represent approximately half of all small molecule drugs approved by the FDA between 1981-2010. These products exhibit massive chemical diversity that even the largest synthetic combinatorial libraries have failed to replicate. Thanks to billions of years of evolution, favourable drug-like properties, such as solubility and high selectivity for specific targets or cells, are inherent. Despite many years of research, only a small fraction of the available natural small molecules have been characterised. For example, the known members of the genus *Streptomyces* have been estimated to produce over 150,000 different metabolites, of which less than 5% have been characterised.
Bactevo has developed a drug discovery platform combining high-density transposon mutagenesis to generate novel chemical diversity in diverse bacterial species (TarGET) and a droplet-based microfluidics platform to allow ultra-high-throughput screening of 1.25 billion mutant clones per day (TrIDENT). The ability of this microfluidics platform to support bacterial and eukaryotic growth in isolation and co-culture allows any ultra-high content screen (UHCS) to be transferred to the TrIDENT platform. Additionally, the 0.5 nL scale of each droplet drastically reduces the number of cells required to directly investigate diseased cells or tissue.
Having validated this platform through the discovery of a novel compound active against Gram-positive pathogens, Bactevo is now utilising their TarGET and TriDENT technologies to focus on a number of therapeutic areas such as Gram-negative infections, rare diseases (MELAS, LHON, Kearns Syndrome) and immuno-oncology.

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The physiological effects of relocating the genes encoding the HU nucleoid associated protein in *Salmonella enterica* serovar Typhimurium.

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Nucleoid associated proteins (NAPs) are trans-acting DNA binding proteins that have a global influence on gene expression. The tight control and differential expression of NAPs is responsible for the fitness of bacteria at various growth phases and under different growth conditions. HU is one of approximately 14 NAPs found in *Salmonella* Typhimurium and it has been shown to play a role in central metabolism, respiration, and virulence. In enteric bacteria, such as *S.* Typhimurium, the HU protein exists as a dimer and is encoded by two closely related genes, *hupA* and *hupB,* that are located in the Ori and NS-right macrodomains respectively. HU subunit composition varies throughout the growth cycle. In early logarithmic phase the α_2 homodimer is predominant. In late logarithmic phase the αβ heterodimer takes over and in stationary phase the β_2 homodimer is found. Understanding the regulation of NAPs, such as the dimeric HU, and their effects globally, on gene regulation is vital for informing our knowledge of synthetic biology. It is also important to consider how the location of a gene on the chromosome can affect downstream pathways, nearby genes, and the overall fitness of the biological system. This will help us not only to create new biological systems but will also allow us to 're-wire' existing biological systems for use in industry. In this study we are investigating the effects of swapping the chromosomal locations of the *hupA* and *hupB* genes including their regulatory regions on the fitness and physiology of *S.* Typhimurium.

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The activator of multiple antibiotic resistance controls genes implicated in biofilm formation

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The multiple antibiotic resistance (*mar*) operon is responsible for resistance to a broad range of antibacterial drugs. As the *mar* operon is conserved across a number of human pathogens, understanding the mechanisms through which it mediates antibiotic tolerance is essential. The *mar* operon is normally transcriptionally silent, but a faulty *mar* repressor (MarR) leads to constitutive expression of MarA (the *mar* activator). This results in the *mar* phenotype of widespread resistance to antibiotics and other environmental stresses including oxidative stress, organic solvents and disinfectants.

The mechanisms through which MarA induces this phenotype are unclear, but many downstream targets of MarA were recently unveiled by a ChIP-seq analysis. We selected one of these targets, *ycgZ*, and verified MarA regulation and binding using promoter-*lacZ* fusions and electrophoeretic mobility shift assays. We found that MarA upregulates transcription of *ycgZ-ymgA-ariR-ymgC* at a site centred 62 nucleotides upstream of the transcription start site. Mutation of the MarA binding site caused complete loss of binding of MarA to the promoter, and a twofold reduction in beta galactosidase activity. YmgA and AriR have previously been shown to indirectly stimulate colanic acid production via the Rcs phosphorelay system. We therefore expect the upregulation of *ycgZ-ymgA-ariR-ymgC* by MarA will result in increased biofilm formation. As such, this work identifies a potential mechanism through which bacteria overexpressing MarA can tolerate antibiotic stresses.

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Molecular insights mechanism of generations of mosaic CTX phages of *Vibrio cholerae* O1 atypical El Tor variants

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Vibrio cholerae, the pathogen responsible for the disease cholera has incredible adaptive capability. Subtle changes in the cholera toxin encoding phages initiate the emergence of novel variants. Some of these variants have the potential to produce elevated levels of cholera toxin and colonize better as compared to the prototype. Pandemic *V. cholerae* strains belonging to the O1 serogroup have two biotypes, classical and El Tor. The sixth pandemic classical biotype *V. cholerae* encoding classical type cholera toxin is now completely replaced by the El Tor biotype strains. More recently, the prototype El Tor strains that produced biotype-specific cholera toxin are now being replaced by atypical El Tor variants harboring classical type cholera toxin encoding phage. We sought to explore the molecular mechanisms leading to the emergence of El Tor strains with the classical type cholera toxin carrying phage. We isolated an intermediary *V. cholerae* strain carrying both El Tor and classical type cholera toxin encoding phages. Our experimental evidence indicates that inter-genomic recombination between two different types of CTX-phages is possible if a host cell is infected by multiple phages. This is a demonstrated instance of how a single segment genome virus re-organizes its genome via recombination between different types of phage genome and leading to generation of new variants of phage and the emergence of atypical El Tor variant cholera pathogen.

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A sticky problem: identifying global regulators of biofilm formation in Pseudomonas aeruginosa

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The gram-negative pathogen Pseudomonas aeruginosa is ubiquitous within the environment and is major cause of lung infection in people with cystic fibrosis (CF). P. aeruginosa rapidly colonises the infant CF lung, where the bacteria form biofilms and exhibit enhanced antibiotic resistance making the infection almost impossible to eradicate. Currently there is limited understanding as to what molecular factors trigger biofilm formation by P. aeruginosa.
PslA encodes the first gene in a biosynthetic cluster responsible for making the polysaccharide "glue" which holds the biofilm together, whereas cdrA encodes a proteinaceous "spar" that helps to cross-link the extracellular matrix. Constructs were made in which the promoter regions of cdrA and pslA were used to drive the transcription of lacZ. These constructs were integrated at a neutral site in the PAO1 chromosome, leaving the endogenous pslA and cdrA loci intact. The resulting strains (which grew blue on XGal plates) were subjected to random plasposon mutagenesis. Colonies which grew pale on XGal-containing plates were further characterized. Phenotypic assays (biofilm formation, virulence factor production and exopolysaccharide synthesis) revealed several mutants that exhibited reduced gene expression as well as decreased biofilm formation. Whole genome sequencing identified gene disruptions and highlighted regulatory roles for several hypothetical genes including a potential sensor kinase, a type 6 secretion protein and an ABC transporter protein. In addition, RNA sequencing data has raised a potential role for two hypothetical proteins, PA1123 and PA2204, in biofilm formation which will also be characterised to investigate their function and involvement in biofilm formation.

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Tracing the ancestry of *Listeria monocytogenes* strains which have genes that cause partial cytosine DNA methylation

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The gram positive organism *Listeria monocytogenes* causes sporadic out breaks of listeriosis and has continued to be a source of worry for the pregnant, elderly and immuno-compromised persons. A discovery at the Food Science Division of University of Nottingham, UK, found that some strains of *L. monocytogenes* possess the cytosine methyl transferase genes and may exhibit a different pathogenic potential. To determine how these strains evolved, lineage analysis was carried out on a collection of *Listeria monocytogenes* strains after which ribotyping by southern hybridization was performed to determine clonality among strains that possess the methylation genes. It was found that the strains with the genes belonged to evolutionary lineage I of *L. monocytogenes*. Furthermore, they had the same ribotype which indicated that they evolved from the same population clone and suggests similar ancestors. This information is important for epidemiologists and it may help in monitoring new emerging strains or species of the organism.

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When replication forks collide - containing fork fusion-mediated pathologies in Escherichia coli

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Chromosome duplication is strictly regulated in all organisms via the assembly of replication forks at origins. In bacteria such as Escherichia coli replication is initiated at a single replication origin (oriC). Two replication forks are recruited and proceed in opposite directions until they fuse opposite oriC in a specialised region that acts as a fork trap, allowing replisomes to enter but not to leave. Our recent work in E. coli has highlighted for the first time that replication fork fusion events can have surprisingly severe consequences. Fork fusion intermediates are processed by an unexpected number of proteins, including RecG, 3' exonucleases, polymerase I and RecBCD. In the absence of correct processing of fork fusion intermediates these persist for longer, triggering the replication restart protein PriA to recruit additional forks, which then replicate an already replicated chromosomal area. The resulting over-replication leads to the formation of double-stranded DNA ends, triggering RecBCD-dependent recombination which, in turn, results in recombination-dependent replication that causes even more over-replication. We can demonstrate that excessive amounts of over-replication are lethal for the cells, highlighting that maintaining accuracy as replication forks fuse is a key event in genome duplication. The consequences of fork collisions illustrate the advantages of the bacterial chromosome arrangement that exploits a single origin, thereby limiting the number of fork encounters to one per cell cycle, and a defined termination area for the safe processing of fork fusion intermediates. Changes to this arrangement cause problems to cell cycle control, chromosome dynamics and cell viability.

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Lessons from genomic and phenotypic characterisation of Corynebacterium diphtheriae strains

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The respiratory disease diphtheria is caused by toxigenic strains of Corynebacterium diphtheriae. A global immunisation programme with toxoid-vaccine has largely controlled the disease; however, several outbreaks have recently been reported from a number of countries. Moreover, non-toxigenic C. diphtheriae strains associated with severe invasive infections are posing a growing threat. A genomic analysis of a collection of C. diphtheriae strains identified a correlation between the number of pilus gene clusters, and gain or loss of gene function at these loci, with the adhesive and invasive properties of strains. Recombination is the major driver behind the evolution and variation in virulence characteristics of C. diphtheriae strains.
C. diphtheriae has been subdivided into four biovars (belfanti, gravis, intermedius and mitis), based on the biochemical characteristics. However, this separation is not supported by our analyses of the genomic variation and the phylogenetic relatedness among strains, except that multiple genes involved in carbohydrate metabolism are absent in biovar intermedius. A high-throughput phenotypic characterisation of representative strains using the Biolog Phenotype MicroArrays system confirmed that intermedius strains have different nutritional requirements compared to other biovars, which maps very well on the genomic analyses. Cell envelope analysis for fatty acid composition revealed very interesting differences that are potentially associated with virulence but not with the biotypes. Therefore, we conclude that biotyping is clinically irrelevant and should be replaced by genomic approaches for a robust and reliable identification of C. diphtheriae strains and their virulence potential.

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The International Pseudomonas aeruginosa consortium: the 1000 plus genomes project

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The International *Pseudomonas aeruginosa* Consortium 1000 plus genomes project consists in collecting and sequencing over 1000 genomes using an analysis pipeline for the study of *Pseudomonas* genome evolution, antibiotic resistance and virulence genes. Metadata, including phenotypic as well as genomic data, for isolates of the collection are provided through the International *Pseudomonas* Consortium Database (IPCD), available at https://ipcd.ibis.ulaval.ca/. Currently the collection contains 1565 *P. aeruginosa* isolates, generously provided by an international community of research scientists from 31 institutions, spanning 125 years back to 1880, and covering about 35 countries, on 5 continents. The strain collection was assembled with the aim of representing maximal genomic diversity. To this end, various criteria were taken into consideration, including geographic origin, previous genotyping, phenotype, and *in vivo* behavior. To date almost 1000 genomes were sequenced and analyzed. Results confirmed 3 major groups of *P. aeruginosa* strains and demonstrated the existence of new sub-groups with as yet unmatched resolution. Our approach will allow us to draw potential links between environmental strains and those implicated in human and animal infections, understand how patients become infected and how the infection evolves over time as well as identify novel therapeutic targets and prognostic markers for better evidence-based decisions on patient care.

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Excision and integration of genomic resistance islands G08 and G62 (GEIs) of Acinetobacter baumannii

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Genomic islands (GEI) are discrete gene clusters encoding for various functions including antibiotic and heavy metal resistance. This study investigates the functions of two distinct integrase genes in G08 and G62 GEI of Acinetobacter baumannii which are hypothesised to be essential for the integration and excision of the GEIs into the bacterial chromosome.Two-step cloning of the right and left flanks of G08 and G62, including terminal repeats, generated synthetic mini-GEIs on E. coli plasmids. Sub-cloning of the constructs into pWSK129-WH allowed transfer of the constructs also to Acinetobacter. Real-time PCR was used to demonstrate excision, utilizing IPTG-inducible plasmid constructs in E. coli and A. baumannii and after mitomycin C induction of the original chromosomal GEIs. We demonstrate the excision of the aforementioned GEIs under natural and artificial conditions. Using IPTG induction, we demonstrated that the excision and circularization of G08 and G62 was dependent of the respective integrases in both E. coli and A. baumannii and the rate was time dependent. Secondly, natural excision of these GEIs was demonstrated using mitomycin C in the original host. qPCR assays showed the relative quantification of excision and circularization occurrence without induction.

The data evidence the functionality of two integrases in excision and integration of the GEIs G08 and G62 under non-induced and induced conditions both when cloned on plasmids in E. coli, A. baumannii as well as when chromosomally located in their natural host. These recombination events occur at different frequencies resulting in genomes plasticity participating in the spread of resistance determinants.

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MOB-2 an energy amplifier; for DNA mobilisation related to two integrative and conjugative elements in Klebsiella pneumoniae

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The genome of Klebsiella pneumoniae HS11286 carries 9 genomic islands, including two integrative and conjugative elements (ICEs), ICEKpnHS-1 and ICEKpnHS-2; the former belongs to the ICEKp1 family and the latter is currently unassigned. Type IV secretion systems (T4SS) are one of the major modules of these ICEs and are responsible for conjugative transfer of nucleoprotein complexes. Preliminary evidence led us to study the interaction between the two distinct T4SSs, from two different ICEs, in DNA mobilisation. A plasmid containing oriT of ICE1 (pACYC184-oriT1) was constructed to facilitate conjugation assays. Initial work revealed that deletion of ICE2 led to a six-fold reduction in trans-conjugants of the ICE1 marker plasmid. Subsequently we demonstrated that deletion of the mob ortholog in ICE2 (mob-2) produced a similar defect which could be complemented in trans. Further, point mutations in Walker motifs of mob-2 provide additional evidence for a role of its product as a source of energy enhancing ICE-1 mediated conjugative transfer. We also report data on transfer of the native chromosomal ICE-1 and degenerative nature of ICE-2 and discuss the potential mechanisms underpinning our results.

Prokaryotic Microbial Infection Forum

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Targeting quorum sensing system with natural compound for reducing virulence in P.aeruginosa PAO1 - an alternative to antibiotic therapy?

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The indiscriminate use of antimicrobials to combat bacterial infections has led to the evolution of antimicrobial resistance (AMR) in a variety of human pathogens like Pseudomonas aeruginosa. AMR is one of the major challenges threatening human health. Research is now focused on finding alternatives to antibiotics that are not bactericidal and will not select for resistance mechanism. In this study, a plant compound trans-cinnamaldehyde (CA) was screened for its potential as an inhibitor of the quorum sensing (QS) system in P. aeruginosa. The bacteria was cultured in nutrient limiting media both in the presence and in the absence of non-lethal (300µg/mL) concentration of CA. The expression of regulatory QS and virulence associated genes was analyzed by RT-qPCR. Treatment with CA resulted in reduced transcriptional expression of the QS autoinducer synthesis genes lasI (25-fold) and rhlI (10-fold). Reduced expression of lasI and rhlI correlated with a decreased expression of the virulence associated genes lasAB and rhamnolipid genes rhlABC respectively. The reduction in transcriptional expression of quorum sensing genes resulted in a decrease in production of extracellular virulence factors- pyocyanin (32%), protease (65%) and elastase (21%) compared to the untreated PAO1. These results show that CA can interfere with the QS system altering expression of virulence associated genes and extracellular production of virulence factors.

Therefore, the ability of CA to demonstrate anti-QS activities at sub-inhibitory concentration presents itself as an interesting compound in bacterial infection treatment without the risk of selection pressure altering the microbial community.

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Development of a microwave-accelerated real-time potentiometric assay for rapid detection of Mycobacterium abscessus

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M. abscessus is a notorious opportunistic and drug resistant mycobacteria pathogen, commonly isolated from patients with cystic fibrosis. M. abscessus is also involved in other disseminated diseases such as skin infections and chronic meningitis. Currently there is no rapid diagnostic tool for this pathogen. The existing tools (PCR, culture) are expensive, laborious and have long turnaround times. The objective of this study was to assess the applicability of a commercially available potentiometric biosensor (Vantix™ Research system) for rapid (10 minute) detection of M. abscessus DNA. A bioinformatic based approach based on published genome sequences in the NCBI data based was used to design DNA probes which specifically targeted the erm-41 gene of M. abscessus. The ability of our in house microwave based system to liberate target DNA from bacterium was also determined. Using this approach, we were able to distinguish seven clinical isolates of M. abscessus from non-M. abscessus isolates (E. coli, M. smegmatis, S. aureus) within 10 mins. In future studies we will confirm the specificity of these probes and its ability to detect the presence of the pathogen in clinical samples

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Investigation of the Influence of Phase Variation on the Biological Phenotypes, Immunity to and Structure of the Flagella in *Campylobacter jejuni*

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Campylobacter jejuni is a commensal of chickens that is responsible for many cases of food borne gastroenteritis in humans. *C. jejuni* produces bipolar flagella comprised of two structural proteins FlaA and FlaB, which are modified with as many as 19 *O*-linked glycans. The flagellum appears essential for colonization of the chicken gastrointestinal tract and is also a major antigen that elicits protective immunity. Some of the *O*-linked glycans are synthesised by biosynthetic enzymes that undergo phase variation through slipped-strand mispairing in G/C-tracts. Currently, the function of protein glycosylation in *C. jejuni* is unknown but it may contribute to evasion of the host immune response. Therefore, flagellar filament protein (FlaA) would be an excellent target for further investigation. We generated a non-glycosylated FlaA protein by cloning *flaA* gene into an expression vector (pLEICS-1) followed by expression in a bacterial expression system. This recombinant FlaA protein was purified by His tag affinity chromatography and then its antigenicity was compared with modified FlaA protein in lysates of *C. jejuni* by probing Western blots with sera from chickens challenged with *C. jejuni*. The immunoblot results demonstrated that sera reacted strongly with recombinant FlaA but not glycosylated FlaA. No differences were observed in reactivity to the glycosylated form of the protein between two *C. jejuni* strain NCTC11168 variants, a chicken-adapted variant (Ca11168) and hypermotile variant (H11168). We hypothesise that these antibody screening results provide a rationale for the modification of filament flagella with sugar moieties therefore influencing immune recognition by the host.

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Discovery of potential probiotic bacteria to treat bovine mastitis and pig infections caused by *Staphylococcus aureus* and *Streptococcus uberis*

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Background: Use of probiotic bacteria could be a useful alternative approach for managing animal health to help control the (mis)use of antibiotics in agriculture.

Material/methods: Over 1000 different clinical and environmental bacterial isolates were screened for natural inhibitors targeting human/animal pathogenic bacteria using antagonism-based assays. Optimisation of inhibitor production by strain AB39 was performed using different broth media and supplements. Purification was carried out using size exclusion, ion exchange and RP-HPLC columns. In addition, spectrum of activity, physicochemical properties and heat stability of the AB39 peptide was determined. The draft genome was obtained and analysed using BAGEL 3 software to determine putative bacteriocin sequences. Orbitrap Mass-Spectrometry (MS) data were used to interrogate the draft genome.

Results: A high yield of the AB39 peptide was achieved using BHI broth plus 10% serum. Strain AB39 was identified as *Streptococcus gallolyticus*. HPLC purified peptide showed significant stability to heat (80^{o}C for 30 minutes) and proteolytic enzymes and a broad Gram positive spectrum including *Staphylococcus aureus* and *Streptococcus uberis.* Analysis with BAGEL 3 identified several putative bacteriocin loci; one was 90% identical to nisin U, produced by *Streptococcus uberis.* Analysis of MS generated sequence tags confirmed production of the nisin variant, which we have named nisin-U3 with a mass of 3,192.37 Da.

Conclusions: To the best of our knowledge, *S. gallolyticus* never been reported as a causative agent of disease in cows and pigs. *S. gallolyticus* holds promise as a probiotic to prevent bovine mastitis and pig infections caused by all nisin-U3 susceptible bacteria.

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Functional profiling of *Corynebacterium pseudotuberculosis* transcriptome in response to acidic, osmotic and thermal stress

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Corynebacterium pseudotuberculosis is a Gram-positive bacterium with the ability to infect a broad range of hosts, causing for example caseous lymphadenitis in goats, ulcerative lymphadenitis in horses, with some reports of infection in humans. Despite it's veterinary and clinical relevance, little is known about the biological mechanisms related to virulence and persistence under the adverse conditions experienced by the organism in the host. This study was based on RNA-Seq data obtained under acidic, osmotic, and thermal stress conditions. A bioinformatics transcriptome-analysis approach was applied in order to identify key mechanisms which may contribute to the persistence of the species under these adverse conditions and, potentially, to its pathogenicity. The NOISeq R package was used for the differential expression analysis, the STRING database for protein-protein interaction, the Blast2GO software for Gene Ontology biological processes and functions, and the KOBAS 2.0 software for KEGG metabolic pathways. We identified mechanisms reported to be important in virulence and persistence in adverse conditions in other pathogenic microorganisms, but that have previously been unreported for *C. pseudotuberculosis*. Several of the up-regulated sets of genes, such as those involved in purine metabolism and RNA regulation, appear to play an important role in the infection process and persistence of the species in the host. This study sheds new light on key mechanisms induced in *C. pseudotuberculosis* in response to stress conditions, and identification of these may lead to a better understanding of the organism, potentially helping in the further development of new treatments and vaccines.

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Phenotypic characterisation of the Merseyside outbreak invasive Group A Streptococcus emm32.2 strains

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Background

Group A Streptococcus (GAS), also known as Streptococcus pyogenes, is the cause of important human diseases rangingfrom minor conditions such as pharyngitis, to invasive infections such as necrotising fasciitis. Between 2010 to 2012, an unusual GAS outbreak was reported in the Merseyside area which was confined to adults, and had a propensity to occur in IV drug users, the homeless or in alcoholics. These records showed that emm32.2 isolates were involved in this outbreak. The mortality rate associated with the outbreak was reported at 29%.

Aims
This project aims to determine the phenotypic characteristics of the emm32.2 Merseyside 2010-2012 outbreak isolates to determine how differences in bacterial attributes may be linked to differential genomic composition, and may subsequently explain the epidemiological characteristics of this emm subtype.

Methods
Initial investigations consisted of comparing bacterial capsule thickness, complement deposition and biofilm formation of invasive isolates of emm32.2 to other invasive and non-invasive isolates
from different emm types.

Results
Results indicated that there was phenotypic heterogeneity amongst emm32.2isolates. Complement deposition was inversely correlated with capsule thickness; isolates with a thick capsule were less sensitive to complement deposition. Moreover, isolates with thicker capsule were found to be poor biofilm
formers and emm6 isolates exhibited the greatest degree of biofilm formation.

Conclusion
In conclusion, our results show that the level of capsulethickness and biofilm formation are important virulencefactors responsible for invasiveness of emm32.2, other bacterial factors, such as Streptolysin O, are also being investigated to elucidate the full mechanism of invasiveness.

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Characterising the host response to Group A Streptococcal septic arthritis

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Background

Streptococcus pyogenes (Group A Streptococcus) is one of most diverse human bacterial pathogens, and has a varied range of clinical manifestations from non-invasive infections to more serious invasive diseases. Streptococcal infection of the joints is a serious medical emergency that is associated with significant risk of a disabling outcome.

Aims

Establish a clinically relevant murine model of sepsis and septic arthritis to investigate host pathogen interactions and understand the determinants of these different clinical syndromes.

Methods

To examine the host response to infection we set up two models; a sepsis and a novel septic arthritis model. Immune cells were enumerated using FACs analysis from the blood and joints over a time course. The genome sequences of the isolates were used to look for differences that could result in different activation of the immune system.

Results

A reproducible and clinically relevant model of sepsis and arthritis was established. In the arthritis model there was an initial influx of neutrophils and delayed migration of macrophages. Destruction of the joint followed after the innate response corresponding with when symptoms were markedly noted in the mice. Genomic analysis has highlighted differences in the super antigen genes (SpeA) between the bacterial isolates that showed different pathogenicity *in vivo*.

Conclusions

We describe a septic arthritis model using GAS, which can be used to further investigate the mechanism of disease. Initial work suggests bacterial characteristics may be predictive of the clinical syndrome. Research is on-going to look further at the mechanism of pathogenesis within the joint.

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Understanding the ecology and evolution of polymicrobial wound infections

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Chronic wound ulcers (CW) from diabetic patients often become colonised with polymicrobial infections of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These have been suggested to be more virulent, antibiotic resistant and result in worse patient outcomes than mono-infections, but the reasons for this remain unclear. Individual virulence mechanisms of *P. aeruginosa* and *S. aureus* have been widely studied, and several interspecies interactions have also been identified. These studies have mainly focused on using well characterized laboratory strains, but the interactions occurring between bacterial species during actual infection are likely to be different than interactions between reference strains. Reference strains of *P. aeruginosa* effectively kill co-cultured *S. aureus*, but studies have shown that a change in *P. aeruginosa* metabolism present in a cystic fibrosis lineage allows a commensal-like interaction where it benefits from interacting with *S. aureus*.
Here we focus on understanding how the ecology of CW shapes cooperation and conflict between different species. We isolated bacterial populations from five patients and six CW and found that four were colonized with *P. aeruginosa*, and the other two had both species. We found that *S. aureus* and *P. aeruginosa* strains isolated from the same wound can often co-exist when grown in synthetic wound fluid. In contrast, when *P.aeruginosa* and *S. aureus* strains are isolated from different wounds, *P. aeruginosa* often outcompetes *S.aureus*. Our findings are an important first step in understanding how ecology influences the evolution of different species within actual wound infections and how this contributes to these highly antibiotic resistant polymicrobial infections.

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IDENTIFICATION OF HYPOCHLOROUS ACID (HOCL) PROTECTIVE MECHANISMS IN *PSEUDOMONAS AERUGINOSA*

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Background: *Pseudomonas aeruginosa* is an opportunistic bacterium, which causes chronic lung infections in cystic fibrosis (CF) patients. The CF immune response to *P. aeruginosa* is characterised by neutrophil-dominated inflammation, which fails to eradicate infection and causes irreversible lung damage. To combat infection neutrophils produce the potent oxidant hypochlorous acid (HOCl). The ways in which *P. aeruginosa* defends itself against HOCl are poorly understood.

Objectives: To identify mechanisms used by *P. aeruginosa* to protect itself against HOCl.

Methods: We screened 712 regulatory gene mutants of *P. aeruginosa* for altered HOCl susceptibility. Growth (OD600) in the presence of a sublethal concentration of HOCl (300 ppm) was followed over 24 hours (n=3).

Results: The screen identified 12 HOCl-resistant and 16 HOCl-sensitive mutants with consistent phenotypes. These strains had mutations in genes encoding regulators of antibiotic resistance, catabolite repression, motility, methionine biosynthesis and quorum sensing. One HOCl-sensitive strain had a mutation in *PA14_07340*, which encodes an AraC-type regulator and is adjacent to *PA14_07355* a putative peroxiredoxin. In-frame deletion mutants of both *PA14_07340* and *PA14_07355* were confirmed to be HOCl-sensitive. PA14_07340 was not required for protection against the oxidants hydrogen peroxide, methyl-viologen (superoxide generator) or tert-butyl-hydroperoxide. Gene expression assays showed that PA14_07340 up-regulates expression of *PA14_07355* in the presence of HOCl.

Conclusions: We have identified a novel regulator PA14_07340 that specifically protects against HOCl stress. This raises the prospect that this regulator plays a critical role in defending *P. aeruginosa* against neutrophil killing.

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Investigation of the Aat system from Enteroaggregative *E. coli* reveals a novel mechanism for protein secretion.

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Type I Secretion Systems (T1SS) secrete a vast array of proteins and represent one of the simplest forms of bacterial protein secretion, requiring only three proteins to function. T1SS are utilised by many pathogenic bacteria to cause disease. In Enteroaggregative *Escherichia coli* (EAEC) 042, an atypical T1SS has been identified experimentally. This system requires five proteins for effective secretion of dispersin (Aap). The Aat system consists of five proteins AatP, AatA, AatB, AatC and AatD, forming a complex that spans the cell envelope, which is reminiscent of a T1SS. We have found that the Aat system is present in a number of different pathotypes of bacteria including Enterotoxigenic *E. coli* (ETEC), Enteropathogentic *E. coli* and *Yersinia enterocolitica*. We have shown that the Aat system in ETEC H10407 is required for the secretion of CexE, an Aap-like protein. Given the similarity between Aap and CexE, we replaced Aap with CexE on the pAA2 plasmid in EAEC 042. Intriguingly CexE did not complement the aggregative phenotype of Aap knock-out strain and instead we observed a hyper-aggregative phenotype in the CexE knock-in strain of EAEC 042. We further investigated the function of each of the proteins in the Aat System separately and have shown that AatC and AatD are responsible for a post translational modification of Aap that is required for secretion. Thus, we have observed that the Aat system can recognise an Aap/CexE-like protein and performs a post-translational modification before secretion occurs to the cell surface.

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Weapons of tumour mass destruction: *Salmonella* transforms immunce cells to delay tumour growth *in vivo*

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A surge in the number of studies reporting anti-tumour effects of bacterial treatment in *in vivo* cancer models has projected *Salmonella enterica* serovar Typhimurium to the forefront of this burgeoning research field. An attenuated strain of particular promise, SL7207, has been associated with delaying tumour growth and in some cases, eradicating tumours completely. However, the exact mechanisms underlying the anti-tumour effects of this cancer therapeutic remain incompletely elucidated.

Here, we demonstrate the tumour-specific localization of SL7207 in a subcutaneous cancer model *in vivo* when injected intravenously, whilst maintaining the clinical score of the infected animals. We show *Salmonella*-dependent tumour growth delay accompanied by immune cell infiltration with macrophages and neutrophils being of most significance. The *Salmonella* specifically invaded the tumour-associated macrophages and transformed their immunosuppresive phenotype. The transformed macrophages displayed a pro-inflammatory phenotype which have enhanced phagocytic and $CD8^+$ T cell recruitment capacities. There was also decreased viability in *Salmonella*-infected tumours compared to sham infected controls.

These data suggest that *Salmonella* can delay tumour growth through alteration of the tumour-associated macrophage phenotype which may be leading to tumour cell death. There may also be a role for neutrophils which are also recruited to the tumour following intravenous *Salmonella* infection. Greater understanding of the molecular mechanisms involved in the anti-tumour effects of *Salmonella* could allow us to exacerbate the specific anti-tumour factors responsible, to further slow or inhibit tumour progression.

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The Beach Bum Survey: Investigating the association between surfing and gut colonisation by antibiotic resistant bacteria

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Introduction: Antibiotic resistant bacteria (ARB) represent an enormous threat to public health. Understanding the means by which people are exposed to and colonised by ARB, as well as the magnitude of the risk will allow the formulation of effective strategies to reduce or prevent the spread of resistant bacteria. Much research has been done on how people acquire ARB, particularly in healthcare settings. However, very little has been done on the role natural environments play in the spread of ARB to people. Coastal waters are regularly polluted by sewage carrying many ARB, and the densities detected have been high enough to pose an exposure risk to surfers when they swallow seawater.

Methods: In a cross-sectional study, we collected rectal swabs from 273 healthy adults in the UK: 143 regular surfers and 130 non-surfers. We screened the swabs for cefotaxime-resistant E. coli. Polymerase chain reaction was used to determine whether resistant colonies were carrying blaCTX-M, which encodes resistance to multiple beta-lactam antibiotics.

Results: Surfers were approximately three times as likely as non-surfers to be colonised by cefotaxime-resistant E. coli, and more than four times as likely to be colonised by blaCTX-M-bearing E. coli (risk ratio = 4.09, 95% CI 1.02 to 16.4, p=0.046)

Conclusions: For the first time, an association between surfing and gut colonisation by antibiotic-resistant E. coli has been demonstrated. This provides evidence in support of our hypothesis that transmission of these clinically important ARB occurs in coastal waters.

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Transcriptional Analysis of Predation by Myxococcus xanthus

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Background
With antimicrobial resistance rampant in the present era, recent research has looked to microbial predation as a basis for natural product discovery. While numerous predators have been extensively studied, identifying and isolating antimicrobial agents from those predators is demanding. This can be largely attributed to a lack of knowledge of the mechanisms involved in predation.

Methods
To explore the mechanisms of predation we performed mRNA sequencing of Myxococcus xanthus (a myxobacterial wolf-pack predator) and Escherichia coli (prey), as well as predator-prey co-cultures, to identify predation-specific changes in gene expression.

Results Of the 7411 genes in the M. xanthus genome, 68 (0.92%) and 1330 (18%) were differentially expressed when exposed to Live or Dead E. coli respectively. The phosphotransferase pathway was up-regulated in the presence of live E. coli, alongside genes involved in cell wall metabolism. In contrast, amino acid, carbohydrate and fatty acid metabolism pathways were up-regulated in the presence of dead E. coli, alongside many hydrolase-encoding genes. Such observations suggest that predation by M. xanthus involves initially breaking open cells, and then on the release of nutrients from killed prey, inducing expression of several other pathways to assimilate biomass from the prey carcass. Prey responded to predation by up-regulating a variety of stress response genes as well as pathways which could potentially mitigate against predator attack.

Conclusion
Our study proposes the notion of a two-step process during wolf-pack predation, manifested as an initial killing phase followed by a consumption phase.

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The role of indole in antimicrobial resistance and bacterial persister formation in *Escherichia coli*.

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Indole is a widespread biological signalling molecule that affects a range of cellular processes in *Escherichia coli* including plasmid stability, extracellular signalling, virulence control and biofilm formation. Recently, indole has been shown to be involved in the formation of antibiotic persisters as well as being a key player in antimicrobial resistance through upregulation of drug efflux pumps. The aim of this study was to further investigate these two aspects of antibiotic resistance. Wild-type and indole-deficient (*ΔtnaA*; tryptophanase knockout) strains of *E. coli* BW25113 were compared to determine the influence of indole on the minimum inhibitory concentration (MIC) of a range of antibiotics in clinical use. Furthermore, both strains were treated with the same antibiotics at 100x MIC and persister cells were enumerated. Pre-treatment and supplementation of the culture with exogenous indole were used in an attempt to repair the effect of the tryptophanase knockout. A difference in MIC between the strains was observed for a variety of antibiotics, including Aztreonam, Ceftriaxone and Phosphomycin; indicating a role for indole in resistance. For persister formation the results were more complex. During the exponential phase of growth wild-type cultures gave rise to a higher frequency of persisters than tryptophanase knockout cultures. Stationary phase cultures behaved differently, showing a higher frequency of persisters for the tryptophanase mutant. However, colonies formed by persisters derived from the tryptophanase mutant grew more slowly than wild-type persisters after the antibiotic was withdrawn.

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Modulating virulence in *Staphylococcus pseudintermedius* with sublethal concentrations of manuka honey.

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Background: *Staphylococcus pseudintermedius* is an opportunistic pathogen present in healthy dogs and cats but can causes skin and surgical infections. Multidrug resistant strains are commonly isolated and it has now been implicated in human infection. Here the aim was to determine whether medical grade manuka honey could reduce the virulence of *S. pseudintermedius*.

Methods: Twenty two clinical *S. pseudintermedius* isolates were used. The minimum inhibitory concentration of manuka honey was established using the European Committee on Antimicrobial Susceptibility Testing guidelines, a sub lethal concentration of 5% (w/v) was used in all subsequent tests. The antibiotic sensitivity of isolates with and without honey was determined. The effect of honey on auto agglutination was measured; the effect on haemolysis, protease and lecthinase activity was also assessed using 5% sheep blood agar, 5% skim milk agar and 5% egg agar.

Results: The minimum inhibitory concentration was less than 12% (w/v) honey for all isolates. Increased sensitivity to all antibiotics tested was seen with sub lethal honey. Addition of honey led to a significant reduction in auto agglutination ($P<0.05$) for all isolates. Haemolysin and protease activity was significantly reduced ($P=<0.05$) for 9/22 and 8/22 isolates respectively. Isolates did not display lecithinase activity.

Conclusion: Manuka honey inhibits *S. pseudintermedius* at low concentrations. Sub lethal concentrations of honey reduce the activity of excreted virulence factors and have the potential to improve the efficacy of clinically relevant antibiotics; manuka honey could be considered as a topical treatment for multidrug resistant *S. pseudintermedius* infections.

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ANTIMICROBIAL SUSCEPTIVILITY PATTERN OF SOME SELECTED BACTERIAL PATHOGENS OF LOWER RESPIRATORY TRACT TO COMMONLY USED ANTIBIOTICS AT UNIVERSTY OF ILORIN TEACHING HOSPITAL, ILORIN, NIGERIA.

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This study was aimed at investigating the prevalence and antimicrobial susceptibility pattern of some selected bacterial pathogens of lower respiratory tract from patients attending University of Ilorin Teaching Hospital, Ilorin, Nigeria. The study was carried out between December, 2013 and February 2014. Sputum specimens were collected from patients and cultured on Blood agar, Chocolate agar and Mac Conkey agar media. Bacterial isolates were identified by Gram staining, series of biochemical tests and antimicrobial susceptibility test was performed according to Clinical and Laboratory Standard Institute (CLSI) guidelines. Out of 103 specimens studied, 16 bacterial species were isolated, giving a prevalence rate of 15.53%. The isolates includes: Klebsiella pneumoniae (81.25%), Pseudomonas aeruginosa (12.50%) and Klebsiella oxytoca (6.25%) in order of ranking. Thirteen 13 (81.25%) isolates were 92.31% susceptible to Ceftriaxone, Gentamycin, Cefuroxime and Ceftazidine and fifteen 15 (93.75%) isolates were 87.2% susceptible to Gentamycin, Ceftazidine and Piperacillin. Klebsiella pneumoniae was the most susceptible amongst the isolates and Klebsiella oxytoca displayed the highest number of resistance (83.3%) to most of the antibiotics tested except Augumentin in which, it was moderately resistant. In conclusion, Klebsiella pneumoniae was the most commonly recovered organism from patients with lower respiratory tract infection in this centre. Resistance to all tested antibiotics by Klebsiella oxytoca as recorded in this study is of clinical significance, with associated possible treatment failure. On the other hand, Ceftriaxone, Gentamycin, Cefuroxime and Ceftazidine remain useful agents in the management of LRTI in this environment if Klebsiella oxytoca is excluded.

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A study on the differences between the antibiotic susceptibility and virulence patterns of UK and Nigerian MRSA isolates

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Staphylococcus aureus has been identified as the main etiological agent of many infectious diseases in Africa, yet data available about the pathogen are still relatively limited compared with information on the pathogen from developed countries. There is an indication that African S. aureus isolates may be more virulent than their counterparts from other parts of the world; consequently, this study aimed to ascertain if Nigerian Methicillin Resistant S. aureus (MRSA) were more virulent than UK MRSA. Furthermore, this study also aimed to investigate differences in the susceptibilities of the Nigerian and UK MRSA isolates to antibiotics commonly used against MRSA in clinical settings.

The toxic effects of both UK and Nigerian MRSA on KB epithelial and A549 lung cell lines were investigated in lactate dehydrogenase cytotoxicity assays. Antibiotic disc susceptibility tests as well as micro-dilution assays were also performed to ascertain the antibiotic susceptibility patterns of the UK and Nigerian MRSA isolates. Results from both antibiotic susceptibility assays showed that Nigerian MRSA were more resistant to antibiotics than the UK MRSA. Based on the 1-tailed t-test, the toxic effects of live Nigerian MRSA on KB epithelial cells were seen to be significantly greater than the toxic effects of live UK MRSA after both 6 and 24 hrs following infection. Therefore, it cannot be said that Nigerian MRSA are more virulent than their UK counterparts, rather the number of bacteria present at time of infection, site of infection and time play a role in determining the severity of an MRSA infection.

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An evaluation of the Luminex xTAG Gastrointestinal Pathogen Panel assay for the detection of multiple diarrhoeal pathogens in feacal samples in Vietnam

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Diarrhoeal disease is a complex syndrome that remains a leading cause of childhood morbidity and mortality globally. Diagnosing enteric pathogens in a timely and precise manner is important for treatment decisions and informing public health policy; accurate diagnosis is a major challenge in industrialising countries. Multiplex molecular diagnostic techniques may represent a significant improvement over classical approaches. We evaluated the Luminex xTAG Gastrointestinal Pathogen Panel (GPP) assay for the detection of common enteric bacterial and viral pathogens in Vietnam. Microbiological culture and real-time PCR were used as gold standards. Tests were performed on 479 stool samples collected from people admitted to hospital for diarrhoeal disease throughout Vietnam. Sensitivity and specificity were calculated for the xTAG GPP for the seven principal diarehoeal aetiologies. The sensitivity and specificity for the xTAG GPP were >88% for *Shigella* spp., *Campylobacter* spp., rotavirus, norovirus GI/GII and adenovirus compared to microbiological culture and/or real-time PCR. However, the specificity was low (~60%) for *Salmonella* spp. Additionally, a number of important pathogens that are not routinely identified in routine hospital procedures such as *Cryptosporidium* spp. and *Clostridium difficile* were detected. The use of the Luminex xTAG GPP for the detection of enteric pathogens in settings like Vietnam would dramatically improve the diagnostic accuracy and capacity of hospital laboratories, allowing for timely and appropriate therapy decisions as well as a wider understanding of the epidemiology of pathogens associated with severe diarrhoeal disease in low resource settings.

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Liposome associated Mycobacterium glycolipids can modulate HIV-1 trans-infection

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Background: Tuberculosis (TB) is the leading cause of death among HIV-1-infected individuals and where co-infection with Mycobacterium tuberculosis (Mtb) exacerbates the progression of both diseases. HIV-1 targets cells through either direct infection (via CD4 and co-receptor molecules) or via trans-infection where C-type lectins, such as DC-SIGN, capture and transfer virus to susceptible cells. We aimed to study the effects of Mtb glycolipids on HIV-1 trans-infection.

Materials/Methods: Mtb glycolipids from pathogenic (EU127, H37Rv, HN878, CDC1551) and non-pathogenic (BCG, MC^{2}155) strains were integrated into liposomes containing cholesterol (Ch) and phosphatydilcholine (PC): PC:0.2Ch:TB ratio. The Raji-DC-SIGN cell line, immature DCs (iDCs) and mature DCs (mDCs) were tested in capture/transfer of HIV-1 pseudo-typed virus particles to TZM-bl cells and where cells have been pre-incubated with liposomes containing Mtb glycolipids.

Results: Trans-infection of HIV-1 via Raji-DC-SIGN cells is differentially modulated depending on the liposome: the efficacy of capture/transfer when Raji-DC-SIGN were pre-incubated with HN878 and CDC1551 liposomes is not changed, whereas EU127, H37Rv, BCG and MC^{2}155 liposomes reduce trans-infection. A similar profile is obtained with capture/transfer via iDCs: suggesting that EU127, H37Rv, BCG and MC^{2}155 liposomes bind to DC-SIGN, blocking capture/transfer via iDCs. The effect on the trans-infection depends on the degree of activation of DCs: capture/transfer via mDCs shows different impacts, suggesting that others mechanisms might be implicated.

Conclusions: Mtb glycolipid liposomes differentially influenced HIV-1 trans-infection via DC-SIGN: depending of the Mtb strain, and the activation of DCs. Further characterisation of TB liposomes will be performed to identify which lipids have an impact.

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Anaplasmosis in buffaloes in Pakistan

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Bovine Anaplasmosis is a tick-borne hemorickettsail disease. It is caused by anaplasma spp., they are more prevelant in tropical and sub-tropical regions of the world. In our study, we designed PCR to detect anaplasma spp. in buffaloes. One hundred blood samples were collected from buffaloes in and around the Lahore city. The stained thin blood films were examined microscopically and 37% blood samples were found positive for intra-erythrocytic bodies which were then selected for DNA extraction. The DNA was extracted using Phenol-Cholorophorm method. Geneious Software was used to allign 16sRNA sequences of anaplasma spp. available in the database of Genbank. Consensus sequence was generated and genus specific primers were designed by using Primer-Blast tool of NCBI. Genus specific primers for anaplasma spp. were used in PCR to amplify 179 bp band. The amplified products were sequenced and blasted with database of Genbank. We observed sequence homology with Anaplasma marginale Anaplasma phagocytophilum. We found 10% and 2% prevalences of Anaplasma marginale and Anaplasma phagoctophilum respectively in buffaloes through PCR.

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The role of Cell Wall Associated Proteins of Staphylococcus pseudintermedius in the Pathogenesis of Skin Disease

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Staphylococcus pseudintermedius is the major cause of pyoderma in dogs and is increasingly being reported as a zoonotic pathogen of humans. Due to the high levels of multidrug resistance and the emergence of methicillin resistant S. pseudintermedius (MRSP), treating recurrent canine pyoderma is becoming increasingly difficult. The critical host-pathogen interactions mediating S. pseudintermedius colonization and infection are not well understood. SpsD and SpsL are cell wall-associated proteins of S. pseudintermedius that display affinity for both fibrinogen and fibronectin. Notably, SpsL has a higher affinity for canine versus human fibrinogen. Here we investigate the role of both proteins in a S. pseudintermedius murine skin abscess infection model. Further experiments identified that mice infected with a deletion mutant of spsD generated skin abscesses equivalent to the isogenic wild type. In contrast, mice infected with a deletion mutant of spsL displayed increased length of surface lesions in comparison to the isogenic wild type and an spsL repaired strain of S. pseudintermedius. Upon histopathology analysis it was observed that mice infected with the deletion mutant of spsL were more likely to develop a regionally extensive inflammation, akin to cellulitis, than a localised focal abscess. Homogenisation of the skin lesion identified that mice with cellulitis had an increased bacterial load in comparison to mice who developed abscesses. In conclusion, SpsL is required for the development of skin abscesses in a murine model of S. pseudintermedius skin infection.

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The *Salmonella* autotransporter, ApeE, is required for gallbladder colonisation and faecal shedding in mice

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Autotransporters, also known as the Type 5 secretion system (T5SS), are some of the most abundantly secreted proteins in Gram-negative bacteria. Most of these proteins studied are shown to be virulence factors. Here, we investigate the role of the *Salmonella* autotransporter ApeE. ApeE is a classical autotransporter (Type Va) and the secreted effector domain contains a GDSL lipase motif. Our previous work demonstrated that ApeE was essential for *Salmonella* Typhimurium survival on tomatoes, but the role of ApeE for infection was previously unknown. To study the role of ApeE virulence, we infected mice with an attenuated *S*. Typhimurium strain, SL3261, and an *apeE* mutant. We show that ApeE is required for long-term bacterial survival (>21 days of infection) in gallbladders of mice. Furthermore, mice infected with the *apeE* mutant tended have reduced faecal shedding in comparison to mice infected with the parental strain. Previous work by Caetano *et al* demonstrated that phospholipid content of mouse bile is reduced upon *Salmonella* infection. Using an *in vitro* growth assay, we show that ApeE is required for *Salmonella* to utilise Lyso-phosphatidylcholine as a sole carbon source. We hypothesise that ApeE is mediating gallbladder survival in mice by hydrolysing bile phospholipids. The breakdown products of these phospholipids could be utilised by *Salmonella* as carbon sources, enabling efficient growth of bacteria. *Salmonella* persistence in gallbladders is believed to be the source of faecal shedding and relapse. Therefore, understanding the role of ApeE in gallbladder persistence may be essential to understanding the phenomenon in typhoid fever.

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The role of antibody in the killing of *Pseudomonas aeruginosa* by healthy control serum.

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Pseudomonas aeruginosa is an opportunistic pathogen that can cause acute or chronic infections in immunocompromised patients. Well known for causing infections in severe burns, the bacteria also causes other acute infections including keratitis and urinary tract infections. Chronic *P. aeruginosa* lung infections occur in patients suffering from cystic fibrosis (CF), bronchiectasis and chronic obstructive pulmonary disorder (COPD). Such infections lead to chronic inflammation, deteriorating lung function and increased morbidity and mortality.

Antibodies normally protect individuals against infection. However, recently we identified that some patients with non-CF bronchiectasis and chronic *Pseudomonas* infection produce O-antigen specific IgG2 that actually protects the colonising bacteria from killing by the immune system. These 'inhibitory antibodies' prevent both serum and cell-mediated killing of the infecting bacterium. During these studies we found that all healthy control serum (HCS) used could kill chronic *Pseudomonas* strains even when those strains were expressing high levels of O-antigen. Here we investigate how HCS kills *Pseudomonas* strains isolated from the environment, acute infections and chronic infections. We found that HCS could kill all chronic strains tested, however acute and environmental isolates were much more variable in their serum resistance profile. HCS was found to have specific antibody, in particular towards outer membrane proteins, for *P. aeruginosa* strains and specific antibody was necessary for the killing of O-antigen positive strains isolated from acute infections. Finally, O-antigen specific antibody purified and concentrated from HCS could inhibit the killing of normal healthy control serum.

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Evolutionary strategies of Bdellovibrio bacteriovorus predators and prey

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Antibiotic resistance is a serious threat to human health and new treatments for bacterial infections are urgently needed. Bacteriophages, which were first used at the beginning of the 20th century, and the predatory bacterium Bdellovibrio bacteriovorus (discovered in 1962) are potential alternatives to antibiotics.

We developed a mathematical predator prey model to compare the effects of Bdellovibrio and bacteriophage on prey bacterial numbers. Our system is a simple chemostat, with an abiotic resource (glucose), a single prey species exhibiting Monod kinetics (E. coli) and up to two predator species with Holling type II functional responses. As Bdellovibrio spends considerable time in the periplasm of its prey as a bdelloplast, this stage is also modelled, giving a delay between prey removal and 'birth' of predators.

Our model showed that larger prey cells were more vulnerable to the effects of predation to the benefit of the predator. We found that too high an attack rate is detrimental for the predator; hence, there was an optimal rate of attack. There was also a distinct difference in effectiveness between Bdellovibrio and bacteriophages. Since many parameters are not well known, we performed a parameter sensitivity analysis. Most changes of parameters had only modest effects, but some had stronger effects on the system dynamics.

Our model showed the potential effectiveness of Bdellovibrio and bacteriophage in combatting bacterial infections and highlights aspects of these systems which need more laboratory study.

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Functional Screening of a Human Saliva Metagenomic Library for Triclosan resistance.

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Triclosan is a broad-spectrum antimicrobial agent which is used widely in both clinical settings and various consumer products such as toothpaste, mouthwashes, and soaps. We wanted to determine if triclosan resistance genes could be isolated from an oral metagenomic library during a functional screen.

To investigate this, a human saliva metagenomic BAC library was constructed in *Escherichia coli* EPI300 using the pCC1BAC vector to avoid culturable bias during screening. Approximately 27,000 clones were screened on LB agar containing 0.06 µg/mL of triclosan (exceeding the MIC of strain EPI300). Multiple triclosan-resistant clones were found. The plasmid from each clone was extracted, retransformed and, if the resistance phenotype followed retransformation, the plasmid was end sequenced. For large inserts, transposon mutagenesis was performed to identify the gene(s) responsible for the phenotype.

The genes conferring triclosan-resistant were identified as *fabI*, which originated from different bacteria. The insert DNA sequence showed homology to DNA from *Neisseria meningitidis, Porphyromonas sp., Campylobacter concises, Campylobacter gracilis, Prevotella sp., and Haemophilus parainfluenzae*. The gene *fabI* encodes an enoyl-acyl carrier protein reductase (ENR), which is essential for fatty acid synthesis in bacteria. Triclosan can bind to ENR and prevent fatty acid synthesis. By introducing the inserts containing *fabI*, ENR was overexpressed in *E. coli*, reducing the inhibitory effect of triclosan.

This study suggests that bacteria might be able to develop triclosan resistance by acquisition of another copy of *fabI* from a heterologous donor.

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Identification of UDP-glucose 4-epimerase (*galE*) Gene Isolating from the Oral Metagenomics DNA Confers Resistance to Quaternary Ammonium Compounds (QACs).

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Use of antimicrobial compounds results in bacteria developing resistance. The human oral cavity is one environment exposed to these compounds through foods and oral hygiene products.

We have adopted a metagenomic approach to screen for resistance genes from both culturable and unculturable bacteria in the human oral cavity. An oral metagenomic library, constructed in Escherichia coli using pCC1BAC vector, was screened against several antimicrobials.

Out of 12,277 clones screened, one clone was found to have resistance against two commonly used antiseptics, cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC). Analysis of the plasmid from this clone showed that it contained a 17.1 kb insert, and the resistance was conferred by a UDP-4 glucose-epimerase (*galE*) gene homologous to one from *Veillonella parvula*. The product of *galE* is involved in LPS production. Analysis of the *E. coli* host showed the cell surface charge was more positive in the presence of *galE*, which could reduce the binding of these positively charges antiseptics to the bacteria.

This is the first time *galE* has been shown to be responsible for resistance against QACs.

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Conditional mecillinam resistance of UTI isolates of *Escherichia coli* when grown in urine.

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Mecillinam is used exclusively to treat Urinary Tract Infections (UTIs). Resistance development in clinical settings has remained low despite the fact that the mutation rate to resistance in laboratory settings is very high since mutations in many genes confer mecillinam resistance (MecR). We have previously shown that *cysB* mutations are the major cause of resistance in clinical isolates.

We grew *cysB* mutants (laboratory and clinical isolates) and other MecR mutants in urine and Mueller Hinton broth (MHB) supplemented with mecillinam. *cysB* mutants are killed at much lower concentrations of mecillinam in urine (0.25 to 1 mg/L) than in MHB (37.5 to 75 mg/L), the other types of MecR mutants are killed at the same concentrations of Mec in urine and MHB (37.5 to 300 mg/L).

Providing *cysB* mutants with cysteine results in loss of the MecR phenotype. We therefore hypothesized that urine contain enough cysteine to abrogate the *cysB* mediated resistance. Urine from 50 people was used as growth medium (-/+Mec) for a clinical *cysB* strain, a *cysB* knock out strain and a wt strain. Metabolome analysis was done on 36 urine samples and all urine samples contained cysteine, which explains the conditional resistance phenotype.

In conclusion, clinical MecR (*cysB*) mutants are not resistant when grown in urine. This suggests that we are currently misdiagnosing UTIs carrying *cysB* mutations as MecR when using the standard clinical laboratory procedures and that mecillinam could probably be used as a treatment also for strains that show a MecR phenotype.

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Reverse vaccinology approach targeting *Staphylococcus aureus* bovine mastitis

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Bovine mastitis is a major economic burden on the dairy industry globally and a significant animal welfare issue. *Staphylococcus aureus* is a leading cause of mastitis and an effective vaccine could have a profound impact in reducing the disease burden. A reverse vaccinology approach was employed to identify vaccine component candidates by comparative genomic analysis of 69 *S. aureus* bovine mastitis isolates representing the breadth of clonal diversity. In total, 62 candidate antigens were selected based on the broad distribution of the gene and ≥ 85% nucleotide homology with the *S. aureus* reference strain (RF122). These genes were cloned and recombinant protein was successfully generated for 43 candidates. 12 proteins exhibited immunological reactivity with serum from convalescent cattle suggesting expression during infection. 40 clinical isolates of bovine *S. aureus* were analyzed *in vitro* for the expression of these antigens using specific antibodies and it was found each isolate expressed at least 5 of the test candidates. Six antigens were purified and used to vaccinate cattle, IgG specific to the test antigens were detected in both the serum and milk of these animals demonstrating the proteins are immunogenic. A challenge model will be employed to determine if a protective immunity can be induced after vaccination with pools of antigens. These data will provide key insights into the feasibility of a *S. aureus* bovine mastitis vaccine designed using a reverse vaccinology approach.

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The Type VI Secretion System in the pathogenesis of Adherent-Invasive *Escherichia coli* strain LF82

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Adherent Invasive *Escherichia coli* (AIEC) is a non-diarrhoeagenic enteric *E. coli* pathotype, which an increasing body of evidence suggests has an aetiological role in Crohn's Disease. AIEC adhere to and invade intestinal epithelial cells, and survive and replicate within macrophages, by subverting host cell processes such as autophagy and phagocytosis. In gram-negative bacteria, invasion and cell host subversion are typically orchestrated by effectors exported by the Type III Secretion System (T3SS), a system notably absent from the AIEC LF82 genome. AIEC effectors are instead thought to be secreted by two Type VI Secretion Systems (T6SS), encoded in the AIEC Pathogenicity Islands (PAI) 1 and 3, termed T6SS-1 and T6SS-3. The T6SS is a bacteriophage tail-like molecular "harpoon," secreting effectors with both anti-eukaryotic and antibacterial activities; facilitating escape from phagocytosis by actin disruption in *Vibrio cholerae*, and niche establishment by killing competing gut bacteria in *Salmonella* Typhimurium. To investigate the role of the T6SS in AIEC pathogenesis, we generated functional knockouts of T6SS-1, T6SS-3, and T6SS-1/3 double mutants in the AIEC strain LF82. The ability of LF82 T6SS-1, T6SS-3, and T6SS-1/3 knockouts to invade and replicate within Caco-2 cells was determined using a gentamycin protection assay. To assess AIEC T6SS-mediated bacterial killing, LF82 T6SS mutants were co-cultured with *E. coli* K12, and K12 survival was quantified. Effector secretion into eukaryotic cells was monitored by a split-GFP assay. We present our latest data on the AIEC T6SSs, their effectors, and their roles in both anti-eukaryotic and anti-bacterial activity of AIEC LF82.

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Phenotyping of Cystic Fibrosis clinical isolates of *Pseudomonas aeruginosa* shows greater between-patient diversity than within-patient diversity

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Introduction

The Cystic Fibrosis lung is a complex environment in which the major pathogen *Pseudomonas aeruginosa* undergoes selection pressures leading to the existence of co-lineages of strains within infected individuals. In this study we tested the hypothesis that within-patient phenotypic diversity of *P. aeruginosa* strains is greater than between-patient diversity.

Methods

641 clinical isolates from 18 chronically infected and 7 recently infected patients were isolated, with on average 24 single isolates per patient sample. These isolates were phenotypically characterised using high-throughput assays including motility, biofilm formation, hydrogen cyanide, pyocyanin and protease production.

Results

A nested ANOVA approach was used to analyse the data and the comparison of the F statistic and its significance was used to determine whether within-patient diversity was greater than between-patient diversity for the phenotypes tested. The data showed both significant within and between-patient variation. However, for all phenotypes, the within-patient variation increased between the early stage and chronic stage of infection. Nevertheless, the overall between-patient diversity was substantially greater than the within-patient diversity. This contrasts what had been previously reported for *P. aeruginosa* epidemic strains isolates.

Conclusion

While within-patient diversity increases from initial to chronic infection, it does not exceed between-patient diversity. Although the specific lung environment and the presence of co-infecting bacteria will be expected to influence the phenotypic diversity of *P. aeruginosa*, our data suggests that the phenotype of the initial infecting strain has a sustained influence on the subsequent phenotypic adaptive trajectory.

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Infectious polyarthirisis: identification of the *Streptococcus dysgalactiae* isolates survival and resistance

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Infectious polyarthritis (joint ill) primarily affects sheep and is mainly seen in lambs up to 4 weeks of age. It manifests as serious joint inflammation leading to lameness, malnutrition and death. It is both a significant economic and health and welfare concern in the sheep industry. The disease is most often associated with the Gram positive bacteria *Streptococcus dysgalactiae* although little is known about the aetiology of the disease or strain variation and specific bacterial virulence determinants.

Streptococcus dysgalactiae strains were isolated from lamb joint fluids from one of the UK farm and compared with an historical strains isolated in the 1980s. Identity was confirmed by 16s rDNA and growth and antibiotic susceptibility characteristics were compared. All *Streptococcus dysgalactiae* strains showed similar growth rates in BHI media, and were able to survive incubation in 0.9% saline for several hours at 37°C, in contrast to our previous studies using phosphate buffered saline as a wash medium. Antimicrobial susceptibility tests were performed with 8 different antibiotics including those routinely used for treatment: erythromycin, oxytetracycline, penicillin V and gentamicin. The results showed that *Streptococcus dysgalactiae* strains were sensitive to these antibiotics but significant variation in resistance profiles for streptomycin and chloramphenicol was observed in comparison with other published studies. Synovial fluids and cartilages were collected from joints post slaughter in order to develop an ex vivo explant culture model. The successful ovine carpal joint model was used for initial investigation of bacterial behaviour within the joint.

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EspF requires the function of other effectors to inhibit EPEC's uptake by J774.A1 macrophages.

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Enteropathogenic E. coli (EPEC) is a non-invasive human pathogen targeting the absorptive epithelia (enterocytes) of the small intestine where it causes diarrhoea, especially in infants. EPEC disease depends on a syringe like structure (a Type Three Secretion System /T3SS) that transfers >20 effector proteins directly into the host cells. The T3SS proteins are encoded by a specific region of bacterial DNA (Locus of Enterocyte Effacement/LEE). The LEE region also encodes transcriptional regulators, chaperones (aid secretion process), the bacterial outer membrane protein Intimin and seven effector proteins (Tir, Map, EspF, EspG, EspZ, EspH and EspB). Delivered effectors subvert a number of host cell functions and signalling pathways, including inhibition of phosphoinositide 3 (PI3) kinase dependent phagocytosis. The latter event is linked to the subversive activities of the EspF, EspH and EspB effectors
Aim: This project aimed to define the molecular details of how EspF inhibits PI3-kinase dependent uptake of EPEC and to investigate the contributions of the EspH and EspB effectors.
Methods
EPEC's anti-phagocytic activity was evaluated by determining the percentage of bacteria internalized by cultured J774.A1 macrophages through immunofluorescence microscopy evaluation. An antibody based approach was used to stain the extracellular bacteria red with total bacteria (extracellular and intracellular) labelled green. The percentage phagocytosed bacteria was calculated as: (Total cell-associated bacteria (Green) minus extracellular bacteria (Red)/Total cell-associated bacteria (Green)) x 100.
Results
We demonstrate that A) EspF is not sufficient to inhibit phagocytosis and B) EspF, Tir and Intimin are responsible for inhibition of bacterial uptake.

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Insights into anti-biofilm activity of antibiotic combinations on bacterial biofilms of *Enterococcus faecalis* using an *in vitro* catheter-associated urinary tract infection model

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Catheter-associated urinary tract infections (CAUTI) are the most prevalent infections caused by enterococci with *Enterococcus faecalis* being the most common causative species.

The aim of this study was to assess the efficacy of first-line antibiotics against clinical strains of *E. faecalis* under conditions mimicking the urinary tract and evaluate potential combinations that could improve the treatment of CAUTI.

Eight *E. faecalis* clinical isolates in planktonic and biofilm states were assessed for susceptibility to ciprofloxacin, nitrofurantoin, trimethoprim and fosfomycin. As a potential avenue to overcome resistance and improve anti-biofilm activity, drug combinations were evaluated for synergy using a chequerboard assay with a pooled human urine medium. Viability of biofilm-associated cells was assessed after a single exposure (24 hours) and multiple exposures (twice daily for 5 days) using clinically relevant antibiotic concentrations. Bacterial quantification was performed by viable counts and staining techniques: resazurin metabolic dye and live/dead stain microscopy.

Fosfomycin + trimethoprim, fosfomycin + nitrofurantoin, ciprofloxacin + trimethoprim combinations were synergistic against planktonic cultures while others were additive. The combination of these synergistic antibiotics led to a 75% decrease in biofilm-associated cell viability compared to exposure to single agents. For the multiple exposures, the fosfomycin + trimethoprim combination was able to significantly ($p=0.001$) maintain its activity against both sensitive and resistant strains.

In conclusion, a fosfomycin + trimethoprim based combination is more effective than single agent therapy and may have the potential to improve the treatment of these recalcitrant infections whilst reducing the selective pressure for antibiotic resistance in enterococci.

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Identify and characterise microRNA active during the Metarhizum anisopliae infection process of Galleria mellonella

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Entomopathogenic fungi, *Metarhizium anisopliae* (Met.) Sorokin, whose natural habitat is soil, have been found to infect a wide range of insect species and offer a biocontrol alternative to chemical pesticides. Understanding the molecular processes regulating infection by such fungi is important in developing targeted and efficient biocontrol products. The discovery of miRNAs in *M. anisopliae* suggests early origins as a gene regulator. MicroRNA could play a significant role in development or growth through modulation of translation and mRNA stability of the target genes. This work aims to identify, quantify, and determine whether microRNA play a role in the regulation of insect infection by the entomopathogenic- fungus *M. anisopliae*. In addition, to determine what genes are targeted by miRNA regulation to better understand their function in fungal development during infection. The experiments to date have focused on developing the methodology for extracting miRNA and total RNA from the *Metarhizium* infection process, sequencing and analysing the samples to identify miRNA. We have compared three commercial miRNA isolation kits for the best performance in extracting miRNA from Galleria mellonella (wax moth) larvae infected with *M. anispoliae followed by sequencing and differential expression analysis of microRNAs during an infection time course*. We identified Metarhizium-specific miRNAs at different stages of infection that align to coding and non-coding regions of the *M. robertsii* geneome sequence.

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Reproducibility of four identification methods of antibiotic-resistant *Mycobacterium tuberculosis* isolated from displaced and nondisplaced Iraqi patients with reference to QuantiFERON

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Background: The first and major step in the diagnosis of TB is its accurate and early detection. To fulfill this objective a number of methods have been developed like acid-fast stain,culture,QuantiFERON and polymerase chain reaction **Methodology**: In the current study there were 50 patients (18 displaced and 32 nondisplaced TB patients) and 40 healthy control. The patient were examined for the presence of TB utilizing QuantiFERON, AFB smear , T.B.culture and primers IS6110 and MPB64. Drug susceptibility of isolates to first-line anti-tuberculosis drugs was performed using the proportion method on Lowenstein Jensen medium (L.J medium) within 2-4 weeks.**Results**: It was found that the frequency of positivity of acid-fast stain,culture , QuantiFERON and primer MPB64 for displaced and non-displaced patients patients was 36 , 33.3 , 100 a 100 respectively and 64 , 66.7 , 100 and100 % respectively too.The present study revealed that 20 isolates out of 34 tested were resistant to one or more of antituberculosis drugs tested which were isoniazid,streptomycin,rifampicin and ethambutol**Conclusions**:The PCR test for the presence of primer MPB64 and QuantiFERON test were 100% positive for all isolates tested .The present study showed that all the mycobacterial isolates tested for antimycobacterial drugs were resistant to at least one antibiotic used and most of them were multiple-resistant.

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Primary respiratory epithelial cell colonisation by non-typeable *Haemophilus influenzae* biofilms induces immune suppression and widespread cytoskeletal modifications

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Non-typeable *Haemophilus influenzae* is an opportunistic pathogen involved in many upper and lower airway diseases including otitis media, chronic rhinosinusitis, chronic obstructive pulmonary disease (COPD), bronchiectasis and cystic fibrosis. COPD is the fourth largest cause of mortality worldwide and NTHi is identified in over 90% of acute exacerbations. The mechanisms by which NTHi makes the transition between asymptomatic colonisation and pathogenicity are poorly understood, however, biofilm formation has been implicated in evasion of host immunity. The aim of this research was to characterize both host and pathogen response to 72h NTHi biofilm formation on cultured primary respiratory epithelium. Label-free LC/MS proteomic analyses identified 411 host proteins in at least 2/3 of the uninfected and infected samples tested. Biofilm formation increased expression of 121 host proteins (>1.5 fold), with significant changes in glycolysis/gluconeogenesis and cytoskeletal organization observed. Furthermore, biofilm formation decreased expression of 51 host proteins (<0.7 fold). These proteins were predominantly involved in cell-cell adhesion, response to chemicals, and cell localization. InterPro analysis of over-represented protein domains also identified decreased expression of the pro-inflammatory S100 proteins which are involved in cytoskeletal remodeling. Thirty-one NTHi proteins were also identified through these analyses. Of the NTHi proteins present in at least 2/3 samples, DnaK and OMP5 were significantly increased in expression and represent promising biomarkers/targets for further investigation. In summary, NTHi biofilm formation results in significant changes in epithelial cell architecture that may be in response to cellular invasion, and may induce immune suppression through decreased expression of S100 proteins.

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Phage-antibiotic synergy has spatio-temporal effects on planktonic and biofilm populations of *Pseudomonas aeruginosa*

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The global threat of antimicrobial resistance is thought to be responsible for over 700,000 deaths per year. Phage therapy - the use of bacteria-specific parasitic viruses to control bacterial infections - may provide a viable alternative to antibiotics. Furthermore, antibiotics and phages have been shown to exert synergistic effects, which suggest that they could be used in combination. Resistant biofilms are responsible for many persistent and chronic infections, yet the combined effects of antibiotics and phages on bacterial biofilms are still relatively unknown. Hence, this study used a microcosm system to specifically focus on investigating biofilm and planktonic populations of *Pseudomonas aeruginosa* treated with combinations of gentamycin and bacteriophage 14|1. We found that in planktonic populations, phage alone had a small effect, antibiotic alone a large effect, and combined therapy had the greatest effect initially, but only an intermediate effect overall, on bacterial densities. Strong synergistic effects were also found in biofilm populations, but in contrast, this effect only became evident during the later stages of the experiment. Morphological changes in biofilm colonies (from large green to small white colonies) were observed across all treatments suggesting bacterial adaptation to spatial environment. Together these results suggest that phage-antibiotic synergy can be effective in controlling both planktonic and biofilm populations of pathogenic bacteria but this effect can vary temporally. These results demonstrate the importance of long-term studies (including evolutionary-timescale) to study phage-antibiotic effects on pathogenic bacteria.

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Characterisation of the inhibition of bovine T and B cells by lymphostatin from attaching and effacing *Escherichia coli*

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Enterohaemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) are enteric bacterial pathogens of worldwide importance. Most EPEC and non-O157 EHEC strains express lymphostatin (also known as LifA), a chromosomally-encoded 365 kDa protein. Lymphostatin is a putative glycosyltransferase that has been shown to be important in the intestinal colonisation of cattle by EHEC serogroup O5, O111 and O26 strains. However, the nature and consequences of the interaction between lymphostatin and immune cells from the bovine host are not well understood. Using purified recombinant protein, lymphostatin was demonstrated to inhibit mitogen-activated proliferation of bovine T cells, and to a lesser extent proliferation of cytokine-stimulated B cells, but not NK cells. Closer scrutiny of its effects on the T cell compartment shows that it inhibits all major cell subsets (CD4, 8, WC-1 and γδ-TCR) and cytokines examined (IL-2, -4, -10, -17A and IFN-γ). Further, T cells are refractory to mitogen for a least 18 hours after transient exposure to protein. Lymphostatin also inhibited activation of T cells stimulated by IL-2 and by antigen presentation using a *Theileria*-transformed cell line and autologous T cells from *Theileria*-infected cattle. Lymphostatin likely acts early in T cell activation, as stimulation of T cells with concanavilin A, but not phorbol 12-myristate 13-acetate combined with ionomycin, was inhibited. Interestingly, ToxB (L7095), a homologue of lymphostatin from *E. coli* O157:H7, was also found to possess comparable inhibitory activity against T cells, indicating a potentially conserved strategy for direct interference in adaptive immune responses by attaching and effacing *E. coli*.

Annual Conference 2017
EICC, Edinburgh
3-6 April

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PATHOGENIC BACTERIA PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF EAR INFECTION AT BAHIR DAR REGIONAL HEALTH RESEARCH LABORATORY CENTER, ETHIOPIA

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Abstract

Ear infection linked with frequent antibiotic prescription, hearing impairment, severe disability and death is a public health threat in developing countries. However, there is scarcity of documented data in the study area. Therefore, this study aimed at determining bacterial etiologic agents and their antimicrobial susceptibility patterns among patients of all age groups referred to Bahir Dar Regional Health Research Laboratory Center. Retrospective data recorded on culture and antimicrobial susceptibility profile were retrieved for analysis. Pus swabs from discharging ears collected and processed for aerobic bacteria culture and susceptibility testing. Of the total 368 pus swab samples processed, 296 (80.4 %) were culture positive. Of which, 289 (97.6 %) were bacteria and 7 (2.4 %) were yeast cells. The proportion of ear infection was higher in males (92.7 %) than females (65 %) (P = 0.014). The frequency of ear infection below

21 years of age was 65.2 %. The predominant isolate was Pseudomonas aeruginosa (29.7 %) followed by Staphylococcus

aureus (26.3 %) and Proteus spp. (21.9 %). High level of antimicrobial resistance rates were observed for amoxicillin/clavulanic acid, ampicillin and penicillin whereas ciprofloxacin, ceftriaxone, chloramphenicol, cotrimoxazole, gentamicin and amikacin were found effective against the isolated bacteria. Aerobic bacterial otitis media linked with high levels of resistance against amoxicillin/clavulanic acid and ampicillin is major health problem in the study area. Moreover, considerable level of oxacillin resistant S. aureus suggests the diffusion of methicillin resistant S. aureus in the community.

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Controlling Dysbacteriosis through Naturally Occurring Bacteriophage in Poultry Intestines

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The intestines of chickens and turkeys are known to be home to a wide diversity of bacteria, the microbiome, colonising shortly after hatch. What is less well known is that there is also a large community of viruses, the virome, found in neonates and some of these viruses are vertically transmitted from the parents into the egg e.g. chicken astrovirus. Early viral infections, including embryonic infections, can result in gut tissue damage that may predispose birds to later bacterial imbalances (dysbacteriosis) thus affecting performance and possibly resulting in specific diseases. We and others have recently discovered by next generation sequencing and metagenomics studies of poultry gut contents, from growth retarded birds, that in addition to these microbiomes and viromes, there is a high abundance of bacteriophage present in commercial poultry suggests a natural control mechanism for bacteria (Devaney et. al, Avian Pathol. 45:616-629 2016). Bacteriophage of the order Caudovirales were detected (Siphoviridae and Myoviridae), with fewer representatives of the Podoviridae, and less still of the Microviridae and unassigned Leviviridae families. The majority of the bacteriophages were identified as targeting E.coli, Enterococcus and Bacteroides species. Identification and culture of specific strains of bacteriophages that have an obligatory lytic stage could facilitate the production of a cocktail of naturally occurring bacteriophages to be administered early in the broiler rearing period before dysbacteriosis occurs. This treatment could be given at 2-3 weeks of age, but after normal enteric bacterial colonisation has been established by day 3 (intestines) and day14 (caeca) post hatch.

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Development of a three-dimensional airway epithelial cell model to study pathogen interactions within the bovine respiratory tract

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Bovine respiratory disease (BRD) complex is a multifactorial infection of cattle that is responsible for substantial economic losses to the livestock industries. Various viral and bacterial pathogens are associated with BRD and the condition is often characterised by a mixed viral-bacterial infection. However, the complex interactions involving bacteria, viruses and the host are poorly understood. Factors contributing to this include the lack of physiologically-relevant and reproducible *in vitro* models and the current reliance on *in vivo* experiments involving cattle. A three-dimensional airway epithelial cell model of the bovine respiratory tract was developed to closely mimic and study the bacterial/viral/host interactions that occur in BRD. Primary bovine bronchial epithelial cells, isolated from fresh abattoir material, were cultivated at an air-liquid interface to stimulate differentiation into typical airway epithelial cells (i.e. ciliated cells, basal cells and mucus-producing goblet cells). The differentiated epithelium displayed tight junction formation and mucocilary activity, hallmarks of the respiratory tract epithelium. The bovine epithelial cell model was successfully colonised by strains of the bacterial pathogen *Mannheimia haemolytica* isolated from diseased cattle and sheep; however, bovine isolates displayed a higher degree of colonisation compared to ovine isolates. Conversely, commensal strains isolated from healthy animals were incapable of colonisation. The model has allowed interactions of *M. haemolytica* with its bovine host to be characterised at the early stages of infection within a physiologically relevant environment. Furthermore, the model has the potential for reducing the number of animals required for pathogenesis studies involving *M. haemolytica* and other bovine respiratory tract pathogens.

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Molecular characterization of virulence factors in *Enterobacter hormaechei* isolated from symptomatic premature babies with sepsis

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Background

Enterobacter hormaechei is most frequently isolated from clinical sources. It has been shown to be of clinical significance by causing nosocomial infections and reported in several outbreaks of sepsis in neonatal intensive care units (NICU). The aim of this study is to determine the resistance to antibiotics and the presence of virulence factors of *E. hormaechei* isolated from symptomatic premature babies in NICU.

Methods

Twenty five strains were isolated from symptomatic premature babies with sepsis from 2007-2015. The isolates were genotyped using pulsed-field gel electrophoresis (PFGE) with the *XbaI* restriction enzyme. Potential pathogenicity-associated traits were determined using specific PCR probes and genomic analysis. Phenotypically antibiotic resistance testing was performed by the Kirby-Bauer method. Isolates were examined for potential, virulence factors, capsule formation, biofilm production, human serum resistance, acid and desiccation tolerance.

Results

The majority of strains were identified as *E. hormaechei* (84%), *E. aerogenes* (16%) and formed unique pulsotypes by using the *XbaI* restriction enzyme. Adhesion genes *papA, papD, fimA, fimC, fimD* were detected. Nearly all strains were 100% resistant to ceftazidime, ampicillin, cefotaxime and gentamicin. While, some strains were resistant to imipenem, meropenem and 3rd generation cephalosporin. All strains showed the ability of form significant biofilms at 37 ºC in infant formula compared with 25 ºC. Tolerance of acidic condition, desiccation and resistance to human serum were shown by the majority of strains tested.

Conclusion

The *E. hormaechei* strains exhibited features that allowed them to survive in the host and resist antimicrobial therapy.

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Trends in the phase variable Type I Restriction modification locus *spnIII* during pneumococcal colonisation of the human nasopharynx.

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SpnIII is a phase variable Type I Restriction Modification system (RM system) found in the core genome of S. pneumoniae. This RM system is capable of generating, by recombination on inverted repeats (IR), six different specificity subunits, SpnIIIA-F. In addition to the alleles of the phase variable specificity subunit (hsdS), the *spnIII* locus consists of helicase (*hsdR*) and methyltransferase (*hsdM*) genes and a site-specific tyrosine recombinase (*creX*).

We were given access to samples from the Experimental Human Pneumococcal Carriage (EHPC) project. The *spnIII* expression patterns within the initial inoculums were compared to the cells recovered from healthy volunteers at 2, 7, 14 and 22 days post colonisation. In addition, mathematical modelling was used to determine whether recombination of the locus *in vivo* was stochastic. Overall no distinct trends emerged, suggesting no single *spnIII* variant has a selective advantage the human nasopharynx, however recombination was shown not to be stochastic.

These data confirm what we have previously seen with murine models of infection, that the *spnIII* composition of the initial inoculum significantly impacts the recovered population.

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***In vitro* activity of tedizolid against staphylococcal biofilms**

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Whilst approved for the treatment of skin and soft tissue infections caused by susceptible bacteria, little is known about the efficacy of tedizolid against staphylococcal biofilms. The aim of this study was to compare this second-generation oxazolidinone with other commonly used antibiotics against staphylococcal biofilms under both static and flow conditions.

Following EUCAST guidelines, MIC and MBC (Minimum Inhibitory / Bactericidal Concentration) values of 60 *Staphylococcus aureus* and *Staphylococcus epidermidis* clinical isolates were determined for tedizolid, linezolid, vancomycin and daptomycin. Subsequently, the susceptibility of biofilm-associated cells of 15 robust-biofilm-forming isolates to these antibiotics at a range of supra-MICs was assessed using a 96-well plate-resazurin assay and flowcell system (BioSurface Technologies).

A lower dose of tedizolid (MIC_{90} 0.5mg/l) was required to inhibit the isolates in planktonic cultures compared to linezolid, vancomycin and daptomycin. Against *S. aureus*, 10xMIC tedizolid exerted greater impact on MRSA biofilms than 10xMIC linezolid or vancomycin (67%, 48% and 53% reduction in cell viability, respectively) and against MSSA biofilms (71% cell reduction versus 48% with linezolid). Efficacy was not significantly different than that shown by daptomycin. Regarding *S. epidermidis*, tedizolid displayed a reduced activity compared to daptomycin and vancomycin but greater than linezolid. Activity against a biofilm was validated under flow-conditions mimicking the *in vivo* infection environment.

Tedizolid efficiency against planktonic staphylococci was superior to vancomycin, daptomycin and linezolid and greater than linezolid against pre-formed biofilms. These results suggest that tedizolid could offer an alternative agent for the treatment of serious staphylococcal infections.

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Small colony variants of *Pseudomonas aeruginosa* are attenuated for virulence and pathogenicity, *in vitro* and *in vivo*

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P. aeruginosa is a common pathogen and frequent cause of nosocomial infections, especially in patients with surgical wounds or burns. Small colony variants (SCV) of this bacteria arise in response to stress and are known to persist intracellularly, evading immune clearance. It is believed that SCVs can subsequently revert to a wild-type (WT) like phenotype (revertant; R) in the absence of environmental stress. Using an *in vitro* human skin cell line (HaCaT) damaged by scratching, it was observed that two different SCVs did not impair tissue repair as much as the WT or R strains. This effect was mediated by soluble secreted bacterial components and to a lesser extent by contact with bacterially derived cellular constituents. In an *in vivo Galleria mellonella* infection model SCV-infected larvae had a lower rate of mortality compared to the WT and R strains. We therefore hypothesise that SCVs allow bacteria to survive within a host by attenuation of virulence and by provoking a diminished immune response, and when environmental conditions are favourable, they revert to a more virulent phenotype. It is possible that by this mechanism, recurrent infection post-antimicrobial treatment could occur. For example, in an infected wound treated with topical antimicrobial treatment the SCV phenotype could arise, upon cessation of symptoms, treatment is stopped but SCVs remain subsequently reverting to a WT-like phenotype (R) and causing re-current infection.

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Development of polymer-coated silicone urinary catheters resistant to *Proteus mirabilis* surface adhesion and swarming motility

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Proteus mirabilis is an opportunistic pathogen that causes complicated urinary tract infections (UTIs). *P. mirabilis* cells utilize the swarming behaviour to rapidly migrate across catheters and ascent the urinary tract. *P. mirabilis* employs a wide variety of virulence factors during the initial colonisation. One major virulence factor is urease, an enzyme that hydrolyses urea to free ammonia resulting in subsequent precipitation of calcium and magnesium phosphate. The alkaline condition leads to the formation of kidney stones and the crystalline biofilms that encrust the catheter tubing. Through structure-activity analysis, we have identified a new monomer combination formed from acrylates with hydrocarbon pendant groups that resists adhesion and swarming of *P. mirabilis*. The new coating is non-toxic and prevents attachment of other pathogens including *Pseudomonas aeruginosa*, *Staphylococcus aureus* and uropathogenic *Escherichia coli*. In addition, the microscopic examination of *P. mirabilis* swarming behaviour suggests its ability to sense and respond to surfaces of different chemical composition via an unknown molecular mechanism.

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Stx2a toxin enhances animal-to-animal transmission of PT21/28 EHEC O157

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Enterohaemorrhagic *Escherichia coli* strains often contain multiple Shiga toxin (Stx)-encoding prophages. In the UK, the predominant EHEC O157 phage type (PT21/28), is thought to have evolved from PT32 Φstx2c^{+} strains after lysogeny by Stx2a expressing bacteriophage. Importantly, PT 21/28 strains have been associated with a 'super shedding' (>10^{3} c.f.u/g faeces) phenotype in cattle, a phenotype proposed to account for the high prevalence of PT21/28 strains in cattle and human infections in the UK over the last decade.

We aimed to assess the effect acquisition of Φstx2a by PT21/28 strains had on 'super shedding' and animal-to-animal transmission. In three separate animal trials bovine calfs were challenged intra-gastrically with either strain PT32 10671 (Φstx2c^{+}), PT21/28 9000R (Φstx2a^{+} Φstx2c^{+}) or PT21/28 9000 (Φstx2a *stx2A::ISEc8* Φstx2c^{+}). Bacterial shedding from challenged animals was monitored by faecal counts. At peak shedding, a high shedding challenged animal was moved to rooms housing n = 5 uninfected sentinel calfs. Transmission between challenged and sentinel animals was monitored by faecal counts of sentinel animals.

Animals challenged with PT21/28 strains shed significantly higher levels of EHEC O157 than those challenged with PT32 10671. However, this phenotype was not dependent on Stx2a. Strains PT21/28 9000R and PT21/18 9000 transmitted to 10/10 and 9/10 sentinel calfs, respectively, but no transmission was observed for PT32 10671. Furthermore, a functional Stx2a toxin in 9000R significantly increased daily shedding and the total bacterial load shed by sentinel animals compared with PT21/28 9000. Thus Φstx2a and Stx2a toxin significantly enhance transmission and shedding of PT21/28 isolates.

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Mobile phones as fomites for transmission of multidrug resistant bacteria in hospitals

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Background

Nosocomial infections with multi drug resistant (MDR) bacteria are an increasing challenge facing hospitals in the UK and worldwide. Infection control within the hospital setting is crucial to reducing MDR cases. Very little is published about mobile phones as fomites, and yet mobile phone use within many hospitals is often ubiquitous.

Method

Mobile phones from 65 hospital workers and 75 non hospital workers (control group) were swabbed to test for contamination with MDR *S. aureus* and *Enterococcus faecalis*. Putative isolates were identified using selective media and biochemical tests, and then tested for levels of antimicrobial resistance using the Kirby-Bauer disk diffusion method. A chi squared test was used to look for significant differences between the two groups.

Results

Although the level of *S. aureus* found on hospital and control group phones was similar, *E. faecalis* was more prevalent on hospital phones (62% vs 32% respectively, $p<0.02$). Of the positive *S. aureus* isolates, rates of methicillin resistance (MRSA) were significantly higher on hospital phones (89% vs 24% of isolates, $p<0.005$). Vancomycin resistance (VRSA) was only found on hospital phones (67% of isolates, $p<0.001$).

Vancomycin resistant enterococci (VRE) rates were also significantly higher on hospital phones (80% vs 29%, $p<0.02$). The overall level of MDR (resistance to three plus antibiotics) was significantly higher on hospital phones (84% vs 18%, respectively, $p<0.001$).

Conclusion

These results suggest that mobile phones of hospital staff could be acting as fomites for transmission of MDR infections in hospitals.

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Microwave technology and enzymatic modifications of plant extracts to obtain compounds with antibacterial activity

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Background. The emergent need of new compounds to treat infections caused by antibiotic resistant bacteria increased the interest in plant extracts. One of the "greener" extraction techniques relatively unexplored is microwave.

Methods. We developed a new methodology, combining microwave with enzyme-based system to recover and modify an extract of Origanum vulgare. Conventional soxhlet extraction was also used. The chemical composition of the extracts was monitored through Phytochemicals Screening Assay, HPLC-MS, NMR, XRD and FTIR. Chemical modification of the extracts was performed through the use of transaminase and hydrolases enzyme-based systems and yeast. The antimicrobial activity was tested against MRSA ATCC43300 and Pseudomonas aeruginosa PAO1 by determining the MIC and MBC (broth microdilution method). Cytotoxicity assays were conducted in human healthy bronchial/tracheal epithelial cells and fibroblasts.

Results. The microwave assisted approach allowed for the best phytochemicals families, namely, phenols, flavonoids, flavonols, proanthocyanidins / products yield. Both extracts were active against MRSA with MIC=0.0015g/mL and MBC=0.003 g/mL (soxhlet) and MIC between 0.006 - 0.0017 and MBC between 0.006 - 0.007 g/mL (microwave). The extracts were also active against PAO1, with MIC of 0.003 g/mL and MBC of 0.006 g/mL (soxhlet) and MIC of 0.006 - 0.0017 g/mL and MBC of 0.012 - 0.007 g/mL (microwave). Cytotoxicity in normal human cells was less noticeable when extracts were obtained from microwave extraction.

Conclusion. We developed a new strategy to improve and modify the properties of oregano extracts combining an environmentally friendly microwave extraction with an enzyme-based system, obtaining extracts with antibacterial and anticancer activity.

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The embryonated chicken egg as an alternative infection model for Salmonella

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Traditionally, the Salmonella genus has been divided into non-typhoidal and typhoidal serovars. Non-typhoidal Salmonella typically cause gastroenteritis, while typhoidal Salmonella cause systemic disease. In recent years, invasive lineages of non-typhoidal Salmonella (iNTS) have emerged in Africa; these isolates have been associated with systemic illness in immunocompromised people, with a mortality rate of 20-25%. The mechanisms and virulence factors responsible for invasive disease are unknown, and more infection models are needed to distinguish iNTS-associated Salmonella.

Salmonella research typically relies upon small animal models such as mice, but large animals such as rhesus macaques have also been used. We favour the development of alternative infection models for testing Salmonella virulence to reduce the use of animals for the study of bacterial pathogenesis and to improve data quality by allowing more experimental replication. We have developed an embryonated chicken egg infection model to study the virulence of African Salmonella strains. In this model bacteria are injected into the allantoic cavity of the egg, and certain Salmonella isolates can invade the bloodstream and reach the liver.

We assessed the virulence of African and European Salmonella wild-type strains in comparison with mutants which showed reduced virulence in other infection models. While wild type strains were capable of liver colonisation, attenuated rpoE mutants were impaired.

The embryonated chicken egg has previously been used to study pathogens such as Listeria monocytogenes and Escherichia coli, and now represents a promising new infection model to study the virulence of invasive non-typhoidal Salmonella.

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Examining the development of resistance using antibiotic combinations against Pseudomonas aeruginosa in a model cystic fibrosis sputum medium

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Pseudomonas aeruginosa is the most prevalent pathogen in Cystic Fibrosis (CF) patients, and infection is associated with worse clinical outcomes. P. aeruginosa is highly adaptable due to its large genome, and readily develops antibiotic resistance. There are not many antibiotics used for treatment of P. aeruginosa in CF, and these antibiotics are often used in combination. A recent in vitro study using P. aeruginosa in nutrient broth demonstrated that the combination of ceftazidime and ciprofloxacin resulted in high level resistance to both drugs, as well as cross resistance to meropenem, whilst the combination of ceftazidime and tobramycin resulted in little development of resistance. Here, we have looked into the effect on inhibition of growth and development of resistance of five clinically relevant antibiotics in media that more closely resembles the environment encountered in the CF lung. Populations of the laboratory strain of P. aeruginosa PAO1 will be grown in artificial sputum medium and treated with all combinations of the five antibiotics, from single treatment to the combination of all five, and the inhibition of growth will be measured along with the development of resistant mutants. Ongoing work will look to explore the effects of these combinations on clinical isolates, and test for the presence of any collateral antibiotic sensitivities in resistant isolates that could allow the development of a drug cycling regimen to further minimise resistance. Determining the combinatorial effects of these antibiotics could ultimately lead to improved treatment guidelines for managing P. aeruginosa infection.

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Use of differentiated airway epithelial cell cultures at an air-liquid interface to study colonisation of the ovine respiratory tract by *Mannheimia haemolytica*

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Respiratory tract infections are one of the principle causes of mortality and economic loss in the livestock industry. These infections are caused by a complex of viral and bacterial species. *Mannheimia haemolytica* is the most common bacterial pathogen associated with respiratory disease complex in cattle and sheep. While some information pertaining to the virulence factors of this organism has been obtained from large animal infections, such experiments are costly and ethically questionable. The process of airway tissue colonisation also remains poorly understood. With this in mind, we developed an *in vitro* ovine differentiated airway epithelial cell culture system grown at an air-liquid interface (ALI). Differentiation was characterised and optimised by carrying out extensive time-course studies and comparing a wide variety of growth parameters. The well-differentiated tissue layer contained the full repertoire of relevant cell types present in the *in vivo* airway epithelium and represents a highly suitable model for infection studies. Pathogenic strains of *M. haemolytica* were capable of colonising ovine airway epithelial ALI cultures *in vitro* and interesting insights into temporal aspects of this process were revealed using immunofluorescent staining, scanning electron microscopy and histological analysis. A panel of virulent and avirulent isolates from cattle and sheep were used to demonstrate patterns of colonisation and tissue invasion that were specific to the pathogenic isolates. These findings reveal previously unseen pathogenic mechanisms of this important respiratory pathogen.

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Characterization of a bacteriophage from avian *Staphylococcus aureus* associated with innate immune evasion

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Staphylococcus aureus is an important human and livestock pathogen. An *S. aureus* phage (ϕAvβ) inserted into the chromosome at the beta toxin gene (beta-converting phage) is present in 90% approx. of human strains and contributes to human-specific innate immune evasion. Comparative genomic analysis of *S. aureus* isolates from infected poultry has revealed an avian-specific subfamily of beta-converting phages represented by multiple variants with distinct integrase gene alleles. To investigate the role of the avian beta-converting phages in host-pathogen interactions, an ϕAvβ-deletion mutant in an avian pathogenic *S. aureus* strain was constructed by allele replacement. Compared to the wild type, the ϕAvβ-deficient strain had reduced net intracellular survival and extracellular growth *in vitro* in chicken bone-marrow derived macrophages. We are currently investigating the fate of these strains within the macrophage using GFP-tagged bacteria. Overall, these data will contribute new information relating to the evolution of avian *S. aureus* and mechanisms of bacterial host-adaptation.

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Yersiniosis in zoo and domestic animals

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Outbreaks of disease with high mortality due to Yersinia pseudotuberculosis occur in lower primates in zoos in the UK. Y. pseudotuberculosis is also recognised as a cause of mortality in finches in aviaries. Y. pseudotuberculosis and Y. enterocolitica can be isolated from the faeces of cattle, sheep, goats, deer, pigs, dogs and cats with diarrhoea, although these organisms can also be excreted in the faeces of animals without clinical disease. Y. pseudotuberculosis and Y. enterocolitica also cause zoonotic disease in human beings. Yersinia pestis, the cause of plague, is exotic to the UK, but cases in human beings continue to occur in eastern Africa, Asia, South America and western USA, where rodents are the reservoir and fleas act as vectors. In this study, Y. pseudotuberculosis was isolated from outbreaks of mortality in lower primates in zoos in Scotland, including common marmosets (Callithrix jacchus), squirrel monkeys (Saimiri sciureus) and ring-tailed lemurs (Lemur catta). An outbreak of mortality due to Y. pseudotuberculosis occurred in rock hyraxes (Procavia capensis). Transmission occurs by the faecal-oral route and free-ranging rodents are likely to contribute to contamination in the zoo environment, particularly in winter. Vaccines against Y. pseudotuberculosis are available for use in zoo animals. Using cefsulodin-irgasan-novobiocin (CIN) agar, preliminary results show that Y. pseudotuberculosis and Y. enterocolitica can be isolated from the faeces of 1-5% of dogs and cats with diarrhoea in Scotland. It is concluded that Y. pseudotuberculosis is an important pathogen in lower primates and rock hyraxes in zoo environments in the UK.

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Efficacy of manuka honey against cystic fibrosis-associated *Pseudomonas aeruginosa* grown in a soft tissue infection model.

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Background:

Without doubt, the emergence and growth of antimicrobial resistance in modern medicine, is one of the biggest threats to our society. For those suffering with Cystic Fibrosis (CF), this is nothing new, as the number of effective treatment options for CF-associated chronic lung infections are at a critical level. Manuka honey has been shown to have excellent antimicrobial prowess against chronic wound infections. Here we aim to determine its effectiveness against CF-associated pathogens.

Methods:

Using methods from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and a Soft Tissue Infection Model (STIM) supplemented with an artificial sputum media (ASM), *P. aeruginosa* Reference Panel isolates associated with CF (n = 23) and reference strains (n = 5) were tested for their susceptibility to varying concentrations of manuka honey and commonly used antibiotics.

Results:

Isolates that were shown to be resistant to clinically prescribed antibiotics (ceftazidime, ciprofloxacin, and tobramycin) using the EUCAST methods, were susceptible to manuka honey, even when greatly diluted, with no observable signs of resistance. Using a more clinically relevant model (STIM) designed to produce robust biofilm communities, manuka honey induced multi-log fold reductions across all of the isolates tested (n = 22), whilst completely inhibiting others (n = 6), a feat which clinically relevant antibiotics could not achieve.

Conclusion:

The ability of manuka honey to inhibit CF-associated pathogens with a more relevant biofilm model, paves way for its potential use for the treatment of CF-infections as a potential sinal rinse solution to clear the nasal cavity

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Molecular Characterisation of Virulence and Antibacterial Resistant among *Klebsiella pneumoniae* Isolated from Neonatal with Sepsis

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Background: *Klebsiella pneumoniae* is a major cause of nosocomial bacteraemia, it's responsible for 75% to 86% of *Klebsiella* spp. infections. We aimed to characterize virulence and multidrug resistance among *K. pneumoniae* isolated from neonates with sepsis.

Method: In this study, 22 *Klebsiella* isolates isolated from babies with sepsis, from 2007 to 2015 have been characterized. The isolates were genotyped using pulsed-field gel electrophoresis (PFGE) with *XbaI* restriction digestion. Potential pathogenicity associated traits were determined using specific PCR probes, genome analysis, and *in vitro* tissue culture assays.

Results: PFGE showed *K. pneumoniae* strains 498 and 500 were clustered together, whereas the other strains were all unique. Traits significantly associated with neonatal infection were capsular genes *ugd*, *gal* and *gnd* in all strains, whereas *wcaJ* was in most strains. Adhesion genes found included *fimH* and *mrkD*, siderophores genes *iutA*, *fyuA* and *entAB* were detected. ESBL production (bla_{SHV}, bla_{TEM} and bla_{OXA}) were noted. Phenotypically, all isolates showed ß-haemolytic activity on horse blood. The majority of strains were able to survive in pH 3.5 for up to 2 hours. Biofilm formation and capsule production were detected in all strains, while hypermucoviscosity was observed in 25%. The antibiogram revealed that most strains were resistant to all antibiotics used except ciprofloxacin. All strains were able to attach to the Caco-2 cell line, while only one strain (453) was able to invade the Caco-2 cell line.

Conclusions: The *K. pneumoniae* isolates exhibited features that allowed them to survive in the host and resist antimicrobial therapy.

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Phenotyping and Genotyping of Antibacterial Resistant *Escherichia coli* Isolated from Neonatal with Sepsis

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Background: Neonatal sepsis caused by *E. coli* is a major global health concern. The aim of this study is to determine antimicrobial-resistance and virulence factors of *E. coli* K1 isolated from neonates with sepsis.

Methods: In this study, 29 isolates from premature babies with sepsis during 2007 to 2015 have been characterized. Strains were genotyped using pulsed-field gel electrophoresis (*XbaI* restriction digestion). Potential pathogenicity associated traits were determined using specific PCR probes, genomic analysis, and *in vitro* tissue culture assays. Antibiotic-resistance was assessed using the Kirby-Bauer method, and biofilm formation in TSB was measured by the 96-well microplate crystal violet method.

Results: Characteristics significantly associated with neonatal infection were capsular type K1 5 of 29, S fimbriae 6.9%, type 1 fimbria 20.6%, invasion 13.8% and yersiniabactin 20.6%. Ninety-nine percent of the strains showed resistance to Augmentin, whereas only 7.0% demonstrated resistance to ceftazidime. Extended-spectrum β-lactamase activity were identified phenotypically in 99% strains with resistance to cefotaxime + clavulanate (CTX+CV) and cefpodoxime + clavulanate (CPD+CV). Furthermore, 77% of strains were multidrug-resistant. All strains formed biofilms at 25^{o}C and 37^{o}C. All strains were able to attach to Caco-2 cell line, whereas only two strains, 1983 serotype O13:H4 and 2114 serotype O18:H7, which were able to invade the Caco-2 cell line.

Conclusion: The *E. coli* K1 strains exhibited features that allowed them to survive in the host (capsulation and biofilm) and resist antimicrobial therapy. The multidrug-resistance and expression of genes encoding colonization factors favour the selection and persistence of these strains in hospitals.

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Proteomic Analysis of ST127 Uropathogenic *E. coli* Reveals Distinct Secretomes

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Background: Uropathogenic *Escherichia coli* (UPEC) is the most common cause of urinary tract infection (UTI). Sequence type 127 is one of the most virulent UPEC strains, but little is known about its pathogenic potential. We sought to investigate this using a quantitative proteomics approach.

Material/methods: Three strains of ST127 (EC18, EC41 and SA189) were analysed in Lysogeny broth (LB) media, artificial urine medium (AUM), and in co-culture with uroepithelial cell-line HT1197 for 5 hours. Soluble and insoluble proteins were analyzed. Trichloroacetic acid protein precipitation was performed on co-culture media and digested proteins and peptides were separated on a Dionex Ultimate 3000 RSLC nano flow system and analyzed in an Orbitrap Velos Pro FTMS.

Results: Expression and Gene Ontology Enrichment analysis revealed different proteomic profiles of the strains cultured in LB and AUM. GOE analysis showed upregulation in the pentose phosphate pathway and glycolysis in EC18 (an O-antigen deficient mutant) when cultured in AUM. These two pathways could be important routes of carbon flux through the central metabolic pathways during growth in urine/AUM. Co-Culture of SA189 with HT1197 cells lead to apparent cytotoxic effects in HT1197 cells not seen with other UPEC strains, but MTT analysis indicated HT1197 cells were viable. Analysis of the SA189 secretome revealed highly abundant bacterial proteins, some of which (e.g. aromatic-amino-acid aminotransferase) were uniquely found during co-culture.

Conclusions: Proteomic analysis is helping to understand the pathogenic potential of ST127 UPEC and may facilitate identification of novel diagnostic or therapeutic targets to reduce UTI.

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Tetrasodium EDTA* as an Adjunctive Therapy That Enhances The Anti-Biofilm Ability Of Non-Antimicrobial Wound Dressings

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Aim: There are currently no wound dressings on the market that have been specifically developed to prevent and control biofilms. The aim of this study was to investigate whether the addition of tetrasodium-EDTA (T-EDTA)*, a universally accepted anti-biofilm agent, enhances the anti-biofilm ability of existing fibrous, non-antimicrobial wound dressings.

Methods: Various non-antimicrobial wound dressings (woven and non-woven) were cut into 5cm x 5cm pieces and either soaked or sprayed with various concentrations of T-EDTA* (2%-15%) and air dried. ASTM biofilm models including MBEC model, CDC Bioreactor model and the Drip slide model were used to test anti-biofilm efficacy. *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Candida albicans* biofilms were grown for 48 hours before exposure to T-EDTA* impregnated wound dressings for 24 hours, before determining total viable counts (TVCs).

Results: The application of T-EDTA* impregnated wound dressings resulted in a 3-Log reduction in microbial numbers in both planktonic and biofilm forms when compared to non-antimicrobial wound dressings after 24 hours. Biofilms stained with LIVE/DEAD BacLight were visualised on Zeiss confocal microscopy and following exposure to T-EDTA* impregnated wound dressing, showed microbial cell death after 24 hours.

Conclusions: T-EDTA* as a stand-alone agent is able to enhance the activity of many commercially available non-antimicrobial wound dressings. Utilising both the dressings inherent characteristics and combining this with T-EDTA*, the anti-biofilm efficacy was increased. T-EDTA* represents a potential adjunct therapy that could be used for next generation anti-biofilm management.

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Efficacy of an Innovative Electrolysed Water Irrigating Solution* for the Control of Biofilms In Wounds

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Aim: Biofilms have been identified in the tissues of chronic wounds and subsequently have been hypothesised to be the pathological barrier to successful wound closure. The aim of this study was to assess an innovative electrolysed water irrigating solution* against planktonic microorganisms and biofilms.

Method: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* were cultured in Tryptone Soya Broth (TSB) overnight at 37°C before diluting for biofilm growth. Biofilms were grown for 24, 48 and 72 hours using the MBEC model and the CDC bioreactor model. Biofilms were washed in 0.85% saline before being treated in with varying concentrations of the electrolysed water* (25%, 50%, 75% and 100%) from times ranging between 10 seconds and 24 hours. Following treatment, the antimicrobial was neutralised and the biofilm sonicated for 10 minutes. Total viable counts (TVC) were determined. To assess the effect on planktonic microorganisms, log reduction assays were performed. The electrolysed water irrigating solution* was used to test direct and indirect cytotoxicity using L929 fibroblasts.

Results: The electrolysed water wound irrigating solution* eradicated planktonic microorganisms in <10 seconds. All biofilms were eradicated following a 15-minute treatment with the electrolysed water. Cytotoxicity testing showed slight cytotoxic effects at higher concentrations, however indirect tests showed the 50% solution to be non-cytotoxic.

Conclusion: This study has shown exceptional antimicrobial and anti-biofilm capability of an electrolysed water irrigating solution*, which may be used as an effective method of microbial bioburden control in wound care.

***Suprox**

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The Efficacy of a Next Generation Anti-Biofilm Wound Dressing Against Biofilms and Inflammation

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Aim: The use of smart wound dressings to effectively target biofilms and biofilm-associated inflammation in problematic wounds is needed. The aim of this study was to investigate the efficacy of a newly developed, smart-release anti-biofilm complex incorporated into wound dressings, on biofilms and inflammation.

Methods: The smart anti-biofilm complex* was incorporated into various non-antimicrobial wound dressings (woven and non-woven) at various concentrations of the complex*. The wound dressings were air-dried. *Staphylococcus aureus* and *P. aeruginosa* biofilms were cultured in Tryptone Soya Broth (TSB) overnight at 37°C before diluting for biofilm growth. Biofilms were grown using the biofilm filter model or CDC bioreactor for 48 hours before being treated with a 2 x 2cm hydrated wound dressing, containing the smart complex*. Confocal microscopy was used to determine the viability of microorganisms inside the wound dressings, using LIVE/DEAD BacLight staining. Dressings were used to treat a 3-dimensional biofilm-infected wound model and the subsequent assessment of pro-inflammatory cytokines was performed using ELISA.

Results: Total viable counts showed a Log-6 reduction of *S. aureus* and *P. aeruginosa* planktonic and biofilms were observed following a 24-hour treatment. Furthermore, confocal microscopy of the residual wound dressing-post treatment showed a lack of viable bacteria amongst dressing fibres when compared with the dressing without the smart complex*. Variations in the detectable levels of pro-inflammatory cytokines, including interleukin-6 was determined.

Conclusions: The next generation smart complex* incorporated into various wound dressing types has demonstrated great potential for the management of biofilms and biofilm-associated inflammation.

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A conserved cysteine residue in the *Streptococcus pneumoniae* RitR repressor contributes to oxidative stress responses

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RitR is the receiver protein of a two-component signalling system in *Streptococcus pneumoniae*; however, unlike other receiver proteins, it does not contain an aspartate (Asp) residue. Instead, a conserved cysteine residue (Cys128) has been substituted for the Asp residue and this Cys has been speculated to be important for RitR's role, which includes regulating iron transport (by repression of *piuA* and *piuB*) and the response to oxidative stress. Three strains were constructed in which the Cys residue in RitR had been substituted for serine (Ser), alanine (Ala), or aspartate (Asp); these residues vary in their resemblance to Cys, with Ser the most, and Asp the least, similar to Cys biochemically and structurally. The *in vitro* growth and *piuA* expression in these strains were compared to each other and to wild-type (WT), wild-type complement (*ΔritR::ritR*), and the knockout mutant (*ΔritR*). The knockout and substitution mutants grew poorly in aerobic and high-copper conditions when compared to WT and *ΔritR::ritR* strains. In the absence of copper the C128S strain demonstrated an intermediary phenotype, growing worse than WT and *ΔritR::ritR* strains but better than *ΔritR* and the other substitution strains. When *piuA* expression was measured by RT-qPCR, the C128S strain had higher levels of expression of *piuA* relative to *ΔritR::ritR*, but lower levels relative to C128D and C128A. These findings suggest that the Cys residue in RitR is functionally important in response to high copper and oxidative stress conditions, which can both be found in the nasopharynx and lungs of the host.

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Adaptive response of *Klebsiella pneumoniae* following biocide exposure

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Klebsiella pneumoniae has the potential to cause severe bacterial infections with the increasing prevalence of multidrug resistant strains presenting a significant obstacle to its' eradication. Virulence properties linked to capsular and biofilm formation also increase the survivability of the cells by enabling microbial persistence and resistance to multiple stresses. This increased durability presents a source for recurrent outbreaks in the hospital environment by acting as a reservoir of viable cells even after the application of stringent cleaning practices.

Strategies for infection control include the use of hydrogen peroxide in liquid and vapour forms. H_2O_2 remains effective even at low concentrations, and acts as a biocide through strong oxidising properties, leading to DNA damage, enzyme disruption and the failure of multiple metabolic pathways. The oxidative response induced by H_2O_2 impacts on transcription regulatory pathways mediated via the OxyR and SoxRS regulons in gram-negative bacteria.

Our data shows that in planktonic form, the efficacy of H_2O_2 killing against different strains of *K. pneumoniae* is dependent on both concentration and time. Importantly, this trend is dependent on capsular levels, with strains expressing thicker, more mucoidal capsules offering increased protection against H_2O_2. Transcriptome analyses on both capsular and non-capsular strains of *K. pneumoniae* demonstrate that multiple regulatory pathways are activated following sub-lethal exposure to H_2O_2. Our transcriptome study demonstrates that H_2O_2 exposure elicits a distinct genetic response pattern. Importantly, the key pathways linked to biocide exposure are also associated with the development of antibiotic resistance.

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Inhibitory Effect of Some Selected Herbs on *Candida albicans*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*

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Abstract

This study aims to investigate the inhibitory effects of 4 crude ethanolic extracts of mangosteen peel (*Garcinia mangostana* L.), guava leaves (*Psidium guajava* L.), noni (*Morinda citrifolia* L.) and green tea (*Camellia sinensis*) against *Candida albicans*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The inhibitory effects were determined by disc diffusion method. The result showed that extract of mangosteen peel, noni, guava leaves, and green tea could inhibit
S. aureus and *S. epidermidis* with the range between 8.24±0.14 to 14.85±1.08 mm. The extract of noni showed inhibitory effects against only yeast with an inhibition zone of 21.78±0.61 mm. Therefore, it can be concluded that the extract of noni gave the best ability to inhibit *C. albicans* ATCC 10231 and the extract of green tea showed the best ability to inhibit *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228.

Keywords : Herbs, Inhibitory effect, *Candida albicans* , *Staphylococcus aureus*, *Staphylococcus epidermidis*

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Outer membrane proteins of *Escherichia coli K1 and Cronobacter* ssp influence interleukin 8 (IL-8) production by H4 and Caco-2 human cell lines.

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Interleukin 8 (IL-8) is one of the most effective chemoattractant molecules and plays an important role in inspiration of neutrophils migration to the sites of infection or injury. This cytokine is known to be produced in response to exposure to pathogens or some of their cell wall components such as Lipopolysaccharide (LPS) and flagella. In neonates over expression of IL-8 might led to necrotizing enterocolitis (NEC) and chronic lung disease We investigated the role of purified (OMPs) of selected isolates of *C. sakazakii, C. malonaticus, C. turicensis* and *E. coli* K1 in the inflammatory response of H4 neonatal cell compared with Caco-2 and endothelial cells. The OMPs of 200 ml overnight culture were extracted and incubated with human cells for three hours at concentration of 100Ug/ml and 37^{o}C in 5% Co_2 incubator. Results indicated that OMPs were able to enhance production of IL-8 by both cell lines and up to 2000pg/ml by strains belong *C. sakazakii* and *C. malonaticus.* Ultimately, this is the first report of investigating H4 cell line with OMPs from varied species of genus *Cronobacter*, and IL-8 produced was obviously higher than that obtained in response to same concentration of OMPs and LPS from *E. coli.* However, further investigation is required as few published data available about using of H4 cells in host pathogen interaction, and our primarily study indicated that H4 cells produced IL-8 more than that produced by Caco-2 cell line when exposed to different pathogens or one of their cell wall components .

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Colonisation of Zebrafish Larvae by Vibrio parahaemolyticus

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Vibrio parahaemolyticus causes gastroenteritis through the consumption of contaminated seafood. The bacteria are frequently isolated from wild healthy fish and shellfish. The critical role of Type IV pili and Type III Secretion Systems in the ability of the bacteria to cause disease has been demonstrated in mammalian infection models. In this study a zebrafish model for the investigation of *V. parahaemolyticus* colonization was developed. Zebrafish have become a popular model for infectious diseases in recent years. They are readily available, can be manipulated with relative ease and share immunological characteristics with humans, in particular the innate immune system.

Zebrafish were infected at an early stage of their life cycle. At 2 days post fertilization (dpf) the zebrafish were incubated with human pathogenic RIMD2210633 *V. parahaemolyticus* via immersion. Locomotor activity, development and survival of the larvae were monitored until 5 dpf. Larval development progressed as normal over the course of the experiment. Infected larvae did not exhibit changes in their locomotor activity compared to the uninfected control. On average 85% of the zebrafish survived until 5 dpf (72 h post infection [hpi]). Most deaths occurred 30-46 hpi. *V. parahaemolyticus* colonization of the larvae commenced after 24 hpi, reaching numbers of $1x10^3$-$1x10^5$ cfu/embryo by 72 hpi.

In conclusion, *V. parahaemolyticus* colonises zebrafish larvae via immersion, without significant observable deleterious effects on the host. This study demonstrates the feasibility and suitability of this model for studying *V. parahaemolyticus* colonization.

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Phytocannabinoids suppress the innate response to periodontal bacteria.

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Cannabis use is a dose-related, tobacco-independent risk factor for plaque-induced chronic periodontitis. While the underlying mechanisms are unknown, it is clear that marijuana exerts anti-inflammatory properties that have the potential to dysregulate the innate response to oral bacteria. We set out to determine if, and how, representatives of the three major marijuana-derived phytocannabinoid subtypes (cannabidiol [CBD]; cannabinol [CBN]; and tetrahydrocannabinol [THC]) may suppress the inflammatory response of primary human monocytes to the Gram-negative periodontal pathogen, Porphyromonas gingivalis. Physiologically relevant doses of CBD, CBN and THC each suppressed P. gingivalis-initiated pro-inflammatory cytokine release while enhancing the production of the anti-inflammatory cytokine, IL-10. CBD, CBN and THC also suppressed pro-inflammatory cytokine release from Filifactor alocis- or Treponema denticola-stimulated monocytes. Higher doses of each phytocannabinoid compromised innate cell viability and inhibited the growth of P. gingivalis and F. alocis, relative to unexposed bacteria. T. denticola was resistant to all cannabinoid doses tested (up to 10.0 µg/ml). Loss of function assays indicated that phytocannabinoid-mediated immune suppression acts via cannabinoid receptor 2 (CB2), not CB1, and utilises a PI3K-related mechanism. If these phenomena occur in vivo, then phytocannabinoids could enhance chronic periodontitis, at least in part, by promoting microbial dysbiosis through direct toxic effects on specific oral bacteria, by compromising innate cell vitality and/or through a suppressed innate response to periodontopathogens.

Anaerobes in infection

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SpoVD a penicillin binding protein involved in sporulation and cephalosporin resistance

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Clostridium difficile is an opportunistic pathogen that causes antibiotic associated diarrhoea. The incidence of *C. difficile* infection (CDI) increased dramatically in early years of this century, an epidemic caused by the previously rare ribotype 027. In addition to causing large hospital outbreaks this lineage was also associated with seemingly more severe disease. Ribotype 027 strains have been reported to produce more spores and more toxin, perhaps going someway to explaining the efficient transmission and poor clinical outcome.

We sought to understand the peptidoglycan biosynthetic pathways active in both vegetative cells and during sporulation in order to identify proteins playing a role in the resistance to cell wall targeting antibiotics.

We have identified a total of 11 genes predicted to encode penicillin-binding proteins (PBPs) in the genome of R20291, the UK prototypic ribotype 027 strain. We have deleted the *spoVD* gene, encoding a class B PBP, in *C. diffcile* R20291. The mutant strain showed two strong phenotypes

: a sporulation defect and cephalosporin sensitivity. In addition, we have demonstrated an interaction between the SpoVD and SpoVE that appears to be crucial in both sporulation and cephalosporin resistance.

Anaerobes in infection

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***In vitro* Assessment of Biofilm Formation in Clinical *Clostridium difficile* PCR ribotype 002 from different time lineages**

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Background: *Clostridium difficile* infection (CDI) is the major cause of hospital- acquired diarrhoea around the world.UK prevalence of *C. difficile* PCR ribotype 002 (CD002) has been recently noted, yet the drivers of increased prevalence remain unclear. We assessed the *in vitro* biofilm formation capacity of CD002 between a diverse panel of clinical *C. difficile* strains from the UK and across Europe.

Material/methods: Sixty CD002 were studied: UK isolates from 2007-8 (geographically distinct, N=15), UK isolates from 2011-2013 (19 locations, N=22), and non-UK European isolates from 2012-2014 (N=23, 20 locations). Biofilm formation capacity was quantified using 96-well microtitre plate assay with crystal voilet staining. Biofilm viable cells (spores and vegetative cells) were also enumerated by counting viable cells from biofilms after 3 and 6 days (CFU/mL) of incubation.

Results. Recent UK/non-UK CD002 formed significantly more pronounced biofilms by 3 days than asynchronous UK CD002 ($P<0.001$). Spore counts within boifilms were significantly greater in recent UK/non-UK CD002 than UK CD002 from 2007-8 ($P<0.002$). Spore formation within biofilms increased over time in all CD002 lineages, with more spores observed in 6 day old biofilms of recent UK CD002 strains (2.6×10^6CFU/ml).

Conclusions: Recent CD002 demonstrated elevated spore numbers and 3 day biofilm formation compared to asynchronous UK CD002, which could enhance their survival and transmission early as well as play a role in recurrent CDI. Further assement using other model systems are required to evaluate the phenotypic and genetic characteristics of CD002 that may be associated with its emergence in the UK.

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Prevalance of *Ureaplasma parvum and urealyticum* in Urban Gambian Women

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Urogenital *Ureaplasma* is associated with urethritis, pelvic inflammatory disease, infertility and adverse pregnancy outcomes in sexually active reproductive aged women. However, it is also frequent in healthy women. The aim of this study was to determine the prevalence and species distribution of *Ureaplasma* in symptomatic and asymptomatic reproductive aged urban Gambian women (n =179, 20 - 49 years). A questionnaire was administered and endocervical and high vaginal swabs were collected from each participant for the detection of *Ureaplasma*, *Mycoplasma genitalium*, *Chlamydia trachomatis* by real-time PCR, and *Trichomonas vaginalis* and bacterial vaginosis by microbiological analysis. *Ureaplasma* was identified in 82 (45.8%) women, of which 45% were symptomatic and 48% asymptomatic. The incidence of *Ureaplasma* was higher in the 31-35 age groups 21 (58.3%). 10% of women who were positive for *Ureaplasma* were co-infected with *T. vaginalis*. Bacterial vaginosis was also found in 21% of women with *Ureaplasma* infection. The most common species in both study groups was *Ureaplasma parvum* (81.7%). *Mycoplasma genitalium* and *Chlamydial trachomatis* were not detected in any of the samples. Bivariate analysis shows that infection with *T. vaginalis* and using hormone contraceptives for >10 years are risk factors but not significantly associated with *Ureaplasma* infection ($p > 0.05$). This is the first study carried out in urban Gambia and it shows that > 40% of sexually active reproductive aged women harbour *Ureaplasma* either in their cervix, vagina or both, which if left untreated can lead to ascending infection resulting in gynaecological complications.

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The application of a tiered Multi Locus Sequence Typing approach to the epidemiological investigation of *Dichelobacter nodosus*

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Dichelobacter nodosus (*D. nodosus*) is the causative pathogen of ovine footrot, a disease which has a significant welfare and financial impact on the global sheep industry. Previous studies into the phylogenetics of *D. nodosus* has focused on Australia and Scandinavia, meaning the current diversity in the U.K. population and its relationship globally, is poorly understood. Numerous epidemiological methods are available for bacterial typing, however few account for whole genome diversity or provide the opportunity for future application of new techniques. Multi locus sequence typing (MLST) measures nucleotide variations within several loci with slow accumulation of variation to enable the designation of allele numbers to determine a sequence type. The usage of whole genome sequence data enables the application of MLST, but also core and whole genome MLST for higher levels of strain discrimination with a negligible increase in experimental cost. An MLST database was developed alongside a seven loci scheme using publically available whole genome data from the sequence read archive. Sequence type designation and strain discrimination was compared to previously published data to ensure reproducibility. The addition of multiple *D. nodosus* isolates from U.K. farms allowed for direct comparisons to populations from other countries. The U.K. isolates define new clades within the global population of *D. nodosus* and predominantly consist of serogroups B and H, however serogroups A, C, E, D and I were also found.

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Assessing the fitness of *Clostridium difficile* mutants using Transposon Directed Insertion Site Sequencing (TraDIS)

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Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacterium and the leading cause of antibiotic-associated diarrhoea in hospitals across the globe. Due to lack of genetic tools, little is known about the mechanisms by which the bacterium colonizes and persists within the gut environment; and the signals required by the bacterium for the expression of its toxin genes.

Transposon directed insertion-site sequencing (TraDIS) combines high-density transposon mutagenesis with high-throughput sequencing to allow the identification of essential genes. The technique was applied to a library of 70,000 unique *C. difficile* mutants, to helped identify genes essential for growth under *in vitro* conditions. Here, we further apply the method to assess gene fitness of a pool of *C. difficile* mutants in the mouse gut. Detailed analysis and comparisons between TraDIS experiments performed under different conditions, have given us insights into which genes are required for *C. difficile* growth in the gut environment. Additionally, the analysis also helped identify which specific genes are essential during each stage of infection.

TraDIS studies have provided an insight into genes essential for colonization, survival and pathogenesis of *Clostridium difficile* and may even aid in identifying novel antimicrobial targets. A total of 62 *in vivo* datasets and 16 *in vitro* datasets are being analysed for this project, making it one of the largest studies of its kind.

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Generation of a low passage panel of *Clostridium difficile* reference strains.

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Clostridium difficile is classified as an URGENT Threat Level Pathogen in the USA due to a four-fold increase in mortality associated with infections and the appearance of antibiotic resistant strains. To facilitate preclinical research, BARDA considered there was an urgent need to generate a panel of well characterised, low passage, high provenance, recent clinical isolates of *C. difficile*. PHE won a competitive international grant application to select and generate this panel.

Four isolates were selected from a database of 960 held by the PHE *Clostridium difficile* Ribotyping Network. Selection was based on severity of infection, recent isolation, antibiotic resistance profile, toxin production and representation of the four main PCR-ribotypes considered to be currently circulating in the UK and North America.

Master and Working stocks of four isolates were produced in batch culture. Spore viability (cfu/ml) was conducted on Fastidious anaerobe agar and Braziers Selective medium. A distinctive pattern of spore germination efficiency was observed using different growth media which greatly affected the predicted viable spore count. Further details of the selection process and spore propagation methodology will be presented. Characterisation of the panel was subsequently conducted and will be presented separately (Mathews *et al*, 2016).

This panel of low passage, recent clinical isolates of *C. difficile* strains will be shipped to a US holding facility for subsequent storage and dissemination for future use in BARDA grant awards. PHE has been successful in one of these and will immediately utilise the panel for use in *in vivo* preclinical trials of antibiotics.

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A novel 3D skin culture model to study anaerobic bacterial infection

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Footrot is a highly prevalent skin disease in sheep characterized by the separation of the hoof from the underlying skin. *Dichelobacter nodosus* is an anaerobic bacterium essential to initiate footrot. We aimed to develop a 3D skin model to study the skin infection with *D. nodosus*, which enables the investigation of bacterial invasion into the interdigital skin layers and the early inflammatory response to this infection. Biopsies were taken from the interdigital skin of sheep post slaughter in an abattoir. The viability of the biopsy was assessed by tissue integrity and cell death over 76 hours. Efficiency of infection with virulent and benign strains of *D. nodosus* was quantified in the biopsies by qPCR. *D. nodosus* and total bacteria were localized within the biopsy by Fluorescent *in Situ* Hybridization visualizing *D. nodosus* in the external layers of the epidermis after 28h of infection. Expression of pro-inflammatory cytokines in response to *D. nodosus* infection was measured. Preliminary data suggest that the tissue released pro-inflammatory cytokines in response to bacterial infection. In summary, this system can realistically mimic the interdigital skin providing relevant information on how anaerobic bacteria infect the skin and the early stages of host response. This novel model is the first of its kind for investigating an anaerobic bacterial infection using sheep skin explants in alignment with the 3Rs of animal experimentation.

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Phenotypic and genotypic characterisation of a panel of *Clostridium difficile* reference strains.

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Public Health England, Salisbury, UK

Background: PHE was commissioned by BARDA to develop a panel of well characterised, low passage, *C. difficile* clinical isolates for use in preclinical trials of new drugs or therapies to protect against *C. difficile* infection. All stocks required thorough characterisation. Details and results of the characterisation process will be presented. The selection and production process are presented separately (Ho *et al*, 2016).
Methods: Master and Working stocks of four isolates were enumerated for spore viability (cfu/ml) on Fastidious anaerobe agar (FAA) and Braziers Selective medium. The strains were also tested for the production of toxin A and B by ELISA assay and Vero cell cytotoxicity assays. The stocks were also tested for purity and Gram stain to confirm the presence of *C. difficile* and absence of possible contamination. Genomic DNA was extracted for Whole Genome Sequencing.
Results: ELISA assay confirmed the presence of toxins A and B and biological activity was confirmed in the cytotoxicity assay. Purity plates and Gram stains of selected colonies showed typical *C. difficile* morphology with Gram positive rods and spores. Whole genome sequencing generated 95% coverage (NCBI Bioproject, PRJNA353581, SRX2367106-SRX2367113).
Conclusion; All four sets of master and working stocks of fresh clinical isolates of *C. difficile* were found to produce biologically active toxins A and B and had high monoculture viability. Each strain has been sequenced and the spores stocks are now ready for use in research aimed at regulatory submissions to the FDA.

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Lactobacilli probiotics as a potential control for Avian Intestinal Spirochaetosis

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Brachyspira species, are the causative agents of avian intestinal spirochaetosis (AIS). AIS mainly affects layer hens, decreasing quality and production of eggs, costing the UK poultry industry alone circa £18million p.a. Prevalence of Brachyspira in the UK has increased significantly in recent years, with up to 90% of free range and 74% of caged hens testing positive for Brachyspira Spp. with emerging antimicrobial resistance a concern. Therefore, a better understanding of the pathobiology and novel measures to mitigate this economically important disease are required.
Eight avian Brachyspira isolates were selected and subjected to genetic and metabolic analysis using the Biolog system and next-generation whole genome sequencing. Moreover, to investigate the mechanisms by which lactobacilli may mitigate AIS, sixteen Lactobacillus isolates were isolated from chicken faeces and characterised using 16S rRNA sequencing, biochemistry and next-generation sequencing. Lactobacilli isolates were screened for in vitro antimicrobial activity against Brachyspira. These studies indicated that the cell free supernatant from lactobacilli isolates significantly inhibited Brachyspira growth, independent of pH ($p \leq 0.01$). Furthermore, these isolates were able to physically interact with Brachyspira in order to significantly impair motility ($p \leq 0.05$). Collectively, these data suggest that physical interactions between Lactobacillus and Brachyspira are key factors in mitigation of disease and that secreted compounds from lactobacilli have inhibitory effects against Brachyspira. NMR analysis was used to identify metabolites with the potential to inhibit Brachyspira. In addition to lactic and acetic acid, other metabolites such as acetoin were identified which may have inhibitory potential, although further studies are required.

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Effect of heat on the germination apparatus of *Clostridium botulinum* groups I and II.

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Foodborne botulism is a major public health concern, causing fatality in approximately 10% of cases. *Clostridium botulinum* produces the botulinum neurotoxin, the most potent toxin known, and ingesting 30-100ng of the toxin can be fatal. Spores of *C. botulinum* are extremely heat resistant and a mild pasteurisation treatment is not sufficient to inactivate the spores. Spores that survive the heat treatment may germinate, followed by cell multiplication and neurotoxin formation. The effect of amino acid stimulated germination on strains Eklund 17B (*C. botulinum* group II) and FT29 (*C. botulinum* group I) were established using the Bioscreen method to measure change in optical density. Germination of *C. botulinum* strain Eklund 17B and FT29 was initiated by the germinants L-alanine and L-cysteine and reached completion after 20-hours incubation. Germination was also confirmed by phase contrast microscopy. Preliminary results showed that germination of strain FT29 occurred at a slower rate than strain Eklund 17B. Future experimentation will establish the spore germination pathways in *C. botulinum* groups I and II. Spores of *C. botulinum* group II are damaged by heating to 85°C for 10 minutes, while spores of *C. botulinum* group I are considerably more heat resistant. Physiological and molecular techniques are to be used to explore damage caused to the spore germination apparatus damaged during excessive heating. Understanding the mechanisms involved in spore germination can improve the control of botulinum neurotoxin forming clostridia.

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Exocellular Enzyme Production by Propionibacterium acnes in Association with Lumbar Disc Herniation

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Propionibacterium acnes is a Gram-positive, anaerobic skin commensal, traditionally considered as a microorganism of low virulence. A role for P. acnes in discitis has been proposed, however few studies have characterised virulence factors produced by herniated disc tissue isolates. Production of putative virulence factors has been reported to play a key role in the pathogenicity of P. acnes species. This study screened 67 P. acnes isolates recovered from human spine intervertebral disc material for the production of exocellular enzymes, including: haemolysins, proteases, DNases, and lipases. 30% of isolates were positive for β-haemolysis which was specific to phylotype IA1 and IB isolates. 93% of isolates were positive for protease activity, with no significant association between recA type and protease activity. All isolates were lipase-positive and DNase-negative. This work suggests the potential importance of haemolysin, lipase and protease enzyme production in P. acnes pathogenesis in relation to disc herniation. The application of haemolysis as a rapid clinical marker for the identification of P. acnes isolates belonging to phylotypes IA1 and IB is also suggested.

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Diversity in S-layer types : a possible role in variation of adhesion and TLR4 signalling in Clostridium difficile .

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Clostridium difficile infection (CDI) is the most common cause of antibiotic-associated diarrhoea [g1]in the developed world. Whilst most clinical symptoms have been linked to the production of two exotoxins, correlation between the role of specific genes in adhesion and immunopathology is less clear. Recently the S-layer of the organism has been implicated in both functions. This structure covers the outer surface of the organism and has been associated with several functions in pathogenesis, including structural integrity, adhesion to host tissue and extra cellular matrix (ECM) proteins, and activation of both innate and adaptive immunity via TLR4 signaling. The principal S-layer protein, SlpA, displays sequence diversity between strains, with 13 distinct types identified. This variation in adherence and intensity of inflammation may reflect the S layer type expressed by different C. difficile strains.

Using whole genome data from a collection of 500 clinical isolates of C. difficile we have generated phylogenetic trees that demonstrate the relationship between slpA type and ribotype. Further, we are able to link these strains to clinical information. Using human colorectal cells in culture, and reporter cell lines we have investigated the role of the different SlpA types on both adhesion and TLR4 stimulation. Preliminary data has confirmed a role for SlpA in TLR4 signaling and further study of the capacity of the 13 different S-layer types to activate this pathway is ongoing. These data when linked to patient outcome should provide important insights into the role of S layer variation and disease.

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A preclinical research model of *Clostridium difficile* infection

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Disruption of the normal commensal microbial community in humans has become a hot topic in terms of a potential link to other forms of disease such a Parkinson's and other neurological disease (Sampson *et al*., Cell 167, 1469-1480, 2016). Rodent models have played a pivotal role in this recent link between the microbiome and neurological disease. Disruption of the healthy microbiota can occur through many means but the most clinically relevant is as a consequence of antibiotic therapy. Using pre-treatment of hamsters with Clindamycin, we have developed a model of *C. difficile* infection. There are several forms of therapy being developed to treat or prevent *C. difficile* infection including some which aim to return a dysbiotic microbiota to a more "healthy" state. In order to assess such treatments, PHE developed a surrogate replacement therapy derived from healthy hamsters. Our team demonstrated that such a "positive control" can be stored frozen and used to help link a series of experiments conducted over an extended period of time. The model has also been used to assess the protective potency of antibiotic therapy.

Here we will present data on the development of the model and its utility to assess several therapies aimed at treatment and prevention of *C. difficile* infection. The model will now also be used to evaluate the consequence of antimicrobial therapy to treatments aimed at other infections using a panel of low passage, recent clinical isolates.

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Probing evolutionary relationships and virulence traits of Clostridium perfringens

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Clostridium perfringens, which is known as the causative agent of gas gangrene, is also associated with numerous intestinal diseases including necrotic enteritis in poultry, food poisoning in human adults and necrotising enterocolitis in pre-term infants. Remarkably, little is known about the genetic makeup of this bacterium with only 20 sequenced genomes currently publicly available. Preliminary pan-genomic analysis on these 20 sequenced genomes revealed that core genes only account for <20%, indicating substantial genetic diversity within this pathogen. Thus, to comprehensively analyse this genetic diversity and probe genetic traits related to pathogenesis, we sought to sequence >300 strains of C. perfringens that were isolated from a wide range of environments including soils, animals (poultry and zoo), humans (adults/ infants/ pre-terms), both healthy and diseased states, using Whole Genome Sequencing (Illumina HiSeq/ PacBio platforms). Using the assembled and annotated genome sequences (Velvet/ Prodigal), we performed genome analysis using various in silico tools (including BLAST and Roary) and databases (including NCBI and VFDB) with the aim to characterise key genetic clusters including virulence gene profiles (toxins, antimicrobial resistance, prophage patterns and other novel traits) and evolutionary relationships. Interestingly, initial analysis on 150 isolates indicated conserved presence of the α-toxin and κ-toxin, but a variable pattern for other toxins (e.g. β2-toxin, δ-toxin and θ-toxin) throughout this species. Through probing a significant number of C. perfringens genomes, we seek to gain insights into its phylogenetic relationships and to pinpoint certain novel virulence traits that could be targeted for disease intervention.

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Influence of gut microbiota on *C. difficile* biofilm formation

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Background: *Clostridium difficile* infection (CDI) is the leading cause of hospital-acquired diarrhoea and treatment failure results in high recurrence rates. Biofilm formation is often associated with recurrent and persistent infections and previous data has shown that *C. difficile* interacts with sessile communities. To this end, we assayed the effect of different microorganisms on *C. difficile* biofilm formation.

Methods: Biofilm formation of *C. difficile* in co-culture with several human gastrointestinal microorganisms was quantified using a crystal violet absorbance assay and bacterial enumeration. The biofilm structure was analysed using scanning electron microscopy.

Results: Co-culture of *C. difficile* with either *Staphylococcus aureus* or *Lactobacillus delbrueckii* resulted in a synergistic effect on biofilm formation with a 0.8- to 2.3-fold increase respectively, compared to *C. difficile* mono-culture (p=0.001). Interestingly, no significant increases in bacterial numbers were observed in these synergistic relationships. Whereas co-culture of *C. difficile* with *Bifidobacterium breve* or *Lactobacillus rhamnosus* resulted in an antagonistic effect, significantly reducing the recovery of *C. difficile* from the biofilms by 2- to 2.8- log CFU/ml, respectively (p=0.001).

Conclusion: These results show that gut microbiota have different interactions with *C. difficile* within the biofilm, potentially enhancing *C. difficile* recruitment into biofilms. Understanding microbial interactions that promote or compromise *C. difficile* uptake and survival in a biofilm could lead to a better understanding of recurrent CDI and give rise to alternative therapeutic options of CDI treatment and prevention.

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Investigation of *Clostridium difficile* spore recovery on a variety of solid media

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Background: *Clostridium difficile* infection (CDI) continues to place a huge burden on healthcare facilities worldwide. *C. difficile* spores survive for prolonged periods in the environment and are responsible for CDI transmission, germinating in the gastrointestinal tract to cause disease.

Methods: We analysed recovery of fresh (<30 days old) *C. difficile* spores on different solid media. Spores of five *C. difficile* isolates (RT001,015,020,027,078) were serially diluted on to CCEY and BHI agar with varying concentrations of taurocholate(TC)(0.1-1%), glycine(GLY)(0.4-4%) and lysozyme(L) (5%) and recovery assessed by colony counts.

Results: CCEY alone or BHI agar infused with the 'classical' germinants taurocholate and glycine presents the best mode of recovery for *C. difficile* spores. Inclusion of taurocholate and glycine in the media improved recovery on BHI but not CCEY agar. Recovery on different solid agars differed markedly ($0\log_{10}$-$8.6\log_{10}$CFU/ml. Increased taurocholate concentrations did not increase recovery. Recovery was completely inhibited in BHI and CCEY agar with 1%TC/4%GLY incorporation. Glycine appears to be inhibitory to *C. difficile* spore germination or outgrowth at higher concentrations in both sets of solid media. Decreasing glycine concentrations to 0.8%, recovery increased marginally in CCEY agar ($0\log_{10}$CFU/ml vs ~$3\log_{10}$CFU/ml), and in BHI agar recovery increased substantially ($0\log_{10}$CFU/ml vs ~$8\log_{10}$CFU/ml). Lysozyme appears to have no stimulatory effect on spore recovery.

Conclusion: We present data illustrating a potential novel mechanism of glycine-mediated inhibition of spore germination or outgrowth in *C. difficile*.

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Evaluating the effects of heat on *Clostridium difficile* spore germination

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Background

Clostridium difficile (CD) infection is the main aetiology of nosocomial antibiotic-associated diarrhoea, placing a large burden on patients and healthcare resources. CD spores are extremely resilient, enabling environmental persistence and transmission. Identifying the effects of exogenous heat on spore germination is essential to further understanding CD germination.

Methods

CD spores were heated at 50, 60, 70 and 80°C (0, 15, 30 and 60 minutes) and effect on germination assessed. Briefly, spore preparations (~$6x10^5$) of 5 PCR ribotypes (001, 015, 020, 027 & 078) were heat-treated in PBS, prior to enumeration on CCEYL agar. In addition, 10 minute heat-treated spores were inoculated into BHI broths with 0.1% taurocholate and 0.4% glycine and incubated anaerobically at 37°C for 90 minutes. Spore and total viable counts (TVC) were assessed by serial dilution and ethanol shock (1 hour) on CCEYL agar; all findings were validated by phase-contrast microscopy.

Results

No differences (<0.1$\log_{10}$CFU/ml) in germination were observed between spores heated in PBS at 50, 60, 70°C and untreated control. However, a reduction in spore recovery of ~3.3$\log_{10}$CFU/ml was observed after exposure to 80°C. Comparable trends were observed with additional broth incubations and findings were confirmed by phase-contrast microscopy. TVCs observed at 50, 60 and 70°C were substantially elevated (~3.7$\log_{10}$CFU/ml) over spore counts, indicating germination. In contrast, TVCs for 80°C exposures were equivalent to spore counts (within ~0.3$\log_{10}$CFU/ml), suggesting inhibition of germination.

Conclusions

These data demonstrate that 80°C heat treatment is inhibitory to CD spore germination; it is not known whether this phenomenon is reversible.

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Bacterial Cellulose: a Potential Protective Support for Probiotics in Simulated Intestinal Conditions

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Bacterial cellulose (BC) is a gelatinous membranous biopolymer produced by *Gluconacetobacter xylinus* at the air-liquid interface of bacterial culture. This study aimed to produce BC and investigate its effect as a protective support for *Bifidobacteria* strains in simulated intestinal conditions. BC was produced by fermentation using Hestrin and Schramm medium, statically at 30 °C for 14 days. BC sheets were harvested, washed, purified and freeze-dried and then milled using Pulverisette 14. To investigate the protective properties of BC; a 24-hour culture of each *Bifidobacteria* strain was centrifuged and cell pellets mixed with 5% sterile PBC (powdered BC) and freeze dried. Prepared samples were then dissolved in simulated gastric juice (SGJ), simulated intestinal juice (SIJ) and SIJ with 1% bile salts (SIJ+B). Preparations were incubated in a microaerophilic environment for 4 hours (SGJ) and 3 hours (SIJ and SIJ+B) and viability measured every hour. In SGJ, a 3 Log reduction in viability was recorded for *B. longum* and *B. animalis*, and only a 1 Log reduction was observed for *B. breve*. In contrast, a total loss in viability was recorded for the control samples (cells only) within 2 hours. There was no detrimental effect when cells were exposed to SIJ, however, when cells were exposed SIJ+B, a 2 log (*B. longum, B. breve*) and a 1 Log (*B animalis*) reduction in viability were recorded. Results from this study have demonstrated that BC can be used as a potential protective support for *Bifidobacteria* strains while transiting the upper GI tract.

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Role of root exsudates and two species of Bacillus on the tomato rhizosphere colonisation

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Plant growth promoting bacteria (PGPR) are an indispensable part of rhizosphere. PGPR promote plant growth directly by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. Different strains of *B.subtilis* and *B.amyloliquefaciens* among PGPR play an important role in the rhizosphere to suppress the plant pathogens and thus, it improves the relationship between PGPR and host plant. The colonisation is the most important criterion to PGPR and it influences by different factors: plants species, PGPR and their lipopeptides production, rhizosphere conditions, and root exudates and their compositions.

At this work, we highlighted to study the effect of root exudates, and some of their compositions on the rhizosphere colonisation, in addition, to study the surfactin role on this criterion. Two strains, B.subtilis BBG131, and *B.amyloliquefaciens* FZB42 were chosen to evaluate their behavior in the rhizosphere.

The results showed a significant different in bacterial growth between *B.amyloliquefaciens* and *B.subtilis*. Glucose, sucrose and maltose were the best carbon sources for *B.amyloliquefaciens* FZB42. In all carbon sources the final biomass of *B.subtilis* BBG131 was less than with *B.amyloliquefaciens* FZB42. There was no growth of *B.subtilis* BBG131 with fumaric acid. Root exudates showed the ability to induce bacterial growth for two strains studied. *B.amyloliquefaciens* FZB42 indicated a high ability to colonize the tomato rhizosphere while the surfactin production was low.

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An investigation of the time needed for horizontal gene transfer to occur in oral bacteria.

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Tn*916* is a conjugative transposon which contains the tetracycline-resistance gene *tet*(M). This study aimed to determine how fast transfer of Tn*916* is likely to inhabitants of the human oral cavity, which is the portal to the digestive system. We used *Bacillus subtilis* (BS34A) as a donor of Tn*916*, and six oral *Streptococcus* spp. and *Enterococcus faecalis*, as recipients. The donor *B. subtilis* is considered as transient bacteria within the oral cavity; normally inhabiting soil. We conducted filter-mating assays between these seven donor-recipient pairs in both aerobic and anaerobic conditions which mimic the conditions found in the mouth.

We observed that *B. subtilis* were able to transfer Tn*916* to *S. oralis, S. pyogenes* and *E. faecalis* at frequencies ranging from 1.220×10^{-8} to 6.823×10^{-9} transconjugants per recipient cell within three minutes. Interestingly we could isolate transconjugants only in anaerobic conditions. The presence of the Tn*916* in transconjugants was confirmed by PCR, sequencing and Southern blot hybridisation assay using a fragment of *intTn* as a probe.

In conclusion, three minutes is a sufficient time for Tn*916* to transfer from transient *Bacillus subtilis* to the oral *S. oralis* and *S. pyogenes* and the gastrointestinal *E. faecalis* in an anaerobic environment.

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Enterocin B3A-B3B produced by LAB collected from infant feces: potential utilization in the food industry for *Listeria monocytogenes* biofilm management

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Enterococcus faecalis B3A-B3B produces a bacteriocin B3A-B3B with activity against *Listeria monocytogenes*, *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus* (MRSA), and *Clostridium perfringens*; but not against fungi or Gram-negative bacteria, except for *Salmonella* Newport. B3A-B3B enterocin has two nucleotides different but similar amino-acids content to class IIb MR10A-MR10B enterocin. The predicted molecular mass of B3A-B3B consists of two peptides of 5,176.31 Da (B3A) and 5,182.21 Da (B3B). Importantly, B3A-B3B impeded biofilm formation of the foodborne pathogen *L. monocytogenes* 162 grown on stainless steel. The antimicrobial treatment of stainless steel with nisin (1 mg. ml^{-1} or 16 mg. ml^{-1}) decreased the cell numbers of about 2 logs CFU. ml^{-1}, impeding thereof the biofilm formation by *L. monocytogenes* 162 or its nisin-resistant derivative strain named *L. monocytogenes* 162R. Further the combination of nisin and B3A-B3B enterocin reduced the MIC value requested to inhibit this pathogen grown in planktonic of biofilm cultures.

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Investigation of Neutral lipid production by *Debaromyces hansenii*

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Abstract

The growth rate, osmolyte production and neutral lipid accumulation of the yeast *Debaryomyces hansenii* were measured in response NaCl stress conditions. Cells of *D. hansenii* were grown in YM and minimal media under different concentrations (0, 0.8, and 1.6 M) of NaCl. The amount of total osmolytes increased in high salinity media, especially in YM cultures which were higher than in minimal medium cultures. The NMR analysis demonstrated that glycerol, arabitol, glucose and trehalose were the main intracellular osmolytes present. The percentage of glycerol was notably increased in media with high concentration of NaCl, and it was higher in YM cultures than in minimal cultures. The data on neutral lipids showed that their accumulation in minimal medium was significantly higher than in YM medium for all salt concentrations especially for 0 M. In order to optimize the neutral lipid yield, *D. hansenii* cells were grown in minimal medium with different carbon/nitrogen ratios. The maximum production was in 48:0.5 and 8:0.25 glucose/ammonium sulphate and glycerol/ammonium sulphate ratios respectively. The effect of stress conditions on the fatty acid composition were also investigated by GC-MS analysis of fatty acid methyl esters (FAMEs). The results showed that palmitic, oleic and stearic acid were the main fatty acids present, and their profile did not change in response to the high carbon/nitrogen ratios. Our experiments are continuing to investigate ways of achieving higher neutral lipid production using molecular techniques to knockout the *GUT2* gene.

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Characterising the microbial communities associated with the water distribution system of a broiler farm and their role in *Campylobacter* infection

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Campylobacter (particularly *C. jejuni*) is the most common bacterial pathogen responsible for infectious intestinal disease in the UK. Poultry is the main source of human infection and in spite of the large body of research, the sources of on-farm contamination remain unidentified. In this study the water distribution system of a commercial broiler farm was investigated as a source or vehicle of transmission. Microbial communities within the system were assessed in a 7-week pilot study covering the whole rearing cycle of a single flock. DNA was extracted from weekly biofilm and bulk water samples for 16S and 18S amplicon profiling. Enrichment broth and selective media were used to isolate the pathogen by culture. Prokaryotic and eukaryotic microbial communities follow similar but not identical patterns across the samples. In general, microbial community profiles of water collected from inside the broiler house were considerably different from those of source water. Biofilm and bulk water microbial communities inside the chicken shed also differed from each other, changed across the rearing cycle and were shown to harbour antibiotic resistance genes. *Campylobacter*-specific 16S rRNA reads were detected in weeks 5 and 7 suggesting a possible role of the water distribution system in *Campylobacter* transmission. The emerging pathogen *C. ureolyticus* was also detected by direct PCR. However, no viable *Campylobacter* colonies were isolated from the water, suggesting a viable but non-culturable state. Viable colonies were isolated on *Campylobacter* selective media and identified as another emerging pathogen, *Helicobacter pullorum,* by whole genome sequencing.

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Occurrence of *Blastocystis sp.* among woodland animals in a wildlife park

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Blastocystis is a widespread, anaerobic protist commonly inhabiting the intestinal tract of both humans and animals. At the genetic level, Blastocystis is extremely diverse comprising 17 genetically distinct subtypes (ST). Pathogenicity of this enteric microbe is currently disputed and knowledge regarding its distribution, diversity and zoonotic potential is fragmentary.
In this study, we investigated the prevalence and distribution of Blastocystis in UK woodland animals housed in a conservation park (Wildwood) in Kent. A total of 108 samples were collected from 26 vertebrate species across the park. A combination of cell culturing techniques, microscopy and molecular biology were used to positively identify Blastocystis. The barcoding region of the small-subunit ribosomal RNA (SSU rRNA) was used for molecular identification and subtyping. Over Eighty per cent of the samples were positive for Blastocystis indicating a wide distribution among the animals in the park. Moreover, the majority of animals, positively identified as carriers, were asymptomatic thus reinforcing the idea of its questionable pathogenicity. Interestingly, we identified novel hosts for some Blastocystis subtypes (e.g. ST4 and ST10), which were considered to have narrower animal host specificity, as well as common trends in subtype distribution with ST10 being identified in the majority of the sequenced samples. This study provides the first thorough investigation of Blastocystis prevalence in a wildlife park in UK, which can be used as a platform for further investigations on the distribution of other eukaryotic gut microbes.

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Ethanol production from Organic Municipal Solid Waste (OMSW) using a novel complex microbial community.

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Lignocellulosic ethanol is a sustainable alternative to fossil transport fuels. We propose the assembly of a robust microbial community from natural environments to transform OMSW into EtOH with the manipulation of physical variables as a means to direct this process.

Environments where lignocellulose degradation occurs were sampled and used as inocula in microcosms with OMSW as substrate, cultured at a range of conditions (aerobic vs anaerobic, 4-7 pH values) at 20°C. The fermentation products were monitored using GC-FID. Bacterial DNA was extracted at different time points and Ion torrent was used to sequence the 16Sr RNA amplicon libraries. Pipeline analyses were performed using Mothur and Qiime software.

EtOH was produced by all the inocula with no significant difference when growing under initial aerobic and anaerobic conditions. EtOH was the major product in rumen and sludge inoculated systems, both generating their highest concentrations (~35mM) when growing at different pH values (7 and 5, respectively). After 14 days of incubation, the rumen community was dominated by *Pseudomonas sp.*, a negligible component in the rumen; whereas *Clostridium sp*. was the most abundant OTU in the sludge. Microcosms inoculated with both inocula generated EtOH as the major fermentation product under the range of pH 5 to 7. In this new community *Pseudomonas sp*. and *Clostridium sp.* co-existed being both the most abundant species.

A novel community able to mainly produce EtOH from OMSW was enriched. Forthcoming experiments will inform a model aimed to understand and optimize the performance of the new community.

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Culturable bacterial and fungal numbers in soil following application of anaerobic digestate, cattle slurry, and chemical fertilizer

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The use of anaerobic digestates as fertiliser has been increasingly recognized for the benefits to soil and for the potential to provide essential nutrients for plant growth. Nevertheless, more information is still needed on the effects of anaerobic digestates on soil microorganisms. The objective of this study was to analyze numbers of culturable bacteria and fungi in a grassland soil following application of anaerobic digestates. Treatments were four types of anaerobic digestate, cattle slurry, chemical fertiliser and a control (no fertilisation). A randomized block designed was established with three replicates. Colony forming units (CFU) of bacteria and fungi were analysed. Culturable bacteria and fungi numbers were quantified using selective plating methods. Application of digestate was found to increase bacteria CFUs, with numbers higher in digestate amended plots (3.62 - 8.77 x 10^6 CFU g^{-1} soil) than in plots treated with chemical fertiliser, slurry or nothing (2.03, 2.04, and 1.77 x 10^6 g^{-1} soil, respectively). Similarly, fungal CFUs were highest in digestate-amended plots (2.61 up to 3.08 x 10^5 CFU g^{-1} soil), compare to the control and slurry treatments (7.30 and 8.56 x 10^4 g-1 soil, respectively). Fungal CFUs of chemical fertiliser did not differ from digestates, slurry and control. These results indicate that digestate changes soil microbiology communities in a different way compared to other organic and mineral fertilisers. Trends will be further investigated using molecular profiling techniques on these soils.

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Quantifying microbial community dynamics in agglomerate-scale heap bioleaching of copper sulphides

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Heap bioleaching – the mircobially catalysed dissolution of metals from ores – is an increasingly prominent technology in today's mining industry. The benefits of bioleaching to liberate metals include reduced emissions and energy costs, along with the ability to process low-grade ores and waste rock. This project builds on previous work which examined the microbial ecology of miniature copper sulphide agglomerate-scale heaps throughout a two month colonization period. The findings showed that the leachate is not an accurate proxy for the system as cell numbers were significantly higher in the interstitial and ore-attached phases, and that community dynamics followed different trends; leachate cell numbers plateaued relatively rapidly following inoculation whereas cell numbers in the interstitial and attached phases increased throughout the experiment. However, what was not clear was the extent to which this difference persists in the longer term following heap colonisation. Furthermore, the work was done with a pure culture of *Acidithiobacillus ferrooxidans*, rather than a mixed culture typical of commercial systems. The current study was conducted in similar agglomerate-scale heaps, using a mixed culture. Harvested heaps were examined to provide snapshots of the quantity of mineral-oxidizing microbes, their locations, and the varied ecology at sixteen different time points over six months. Persistent differences in community structure and cell numbers between the leachate and ore-associated phases were observed past the initial colonisation period. Knowledge of microbial distribution and biodiversity in different phases within these systems allows the integration of ecological modelling into understanding and predicting overall heap bioleaching performance.

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Characterisation of a multi-drug resistant plasmid isolated from the gastrointestinal tract of broiler chickens

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Antibiotic resistance is a major problem affecting human and animal health. Plasmid-mediated antibiotic resistance is of particular concern, as many plasmids have the ability to transfer between different bacterial species. This is a threat to human and animal health if a plasmid carrying a resistance gene is transferred to commensal bacteria or clinical pathogens. We investigated antibiotic resistance in the gastrointestinal tract of broiler chickens, which are raised for meat production. There is a possible transmission pathway from animals to humans through food. Antibiotics are used extensively in agriculture. However, an increase in food safety concerns has resulted in banning the use of antibiotics as animal growth promotors in the EU. We identified a multi-drug resistant plasmid from the cecal bacteria of broilers via a direct extraction and plasmid amplification method. The plasmid, which was transformed into *Escherichia coli*, confers high levels of resistance to five different antibiotics: ampicillin, tetracycline, colistin, kanamycin and ciprofloxacin. The resistance to colistin is particularly alarming, as it is considered as an antibiotic of last resort. PCR testing will identify the genes responsible for conferring resistance and the conjugative ability of the plasmid will be assessed. This raises concerns over the control of disease in animals, and food safety, as there is a possibility of the transfer of resistance to humans through food. The plasmid may also have the ability to transfer to other pathogenic Enterobacteriaceae. Infections would be difficult to treat given the multi-drug resistant nature of the plasmid, with limited treatment options available.

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Characterization of multidrug resistance plasmids conferring resistance to colistin in wastewater treatment plant effluent

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The high bacterial loads present in WWTPs facilitate the transfer of plasmids carrying antibiotic resistance genes (ARG) (R-plasmids) between bacteria. Colistin is a last-resort antibiotic for treatments of infections caused by multidrug resistant (MDR) gram-negative pathogens. The aim of this work was to characterise WWTP effluent in Ireland as a source of MDR-plasmids including those conferring colistin resistance. R-plasmids were isolated using two methods: directly from WWTP effluent using an exogenous isolation method; and from bacterial cultures via conjugation and/or transformation. The antibiotic resistance profile of isolated R-plasmids was determined using antibiotic susceptibility tests to identify MDR-plasmids. Extracted MDR-plasmids conferring colistin resistance were tested for the presence of the mcr-1 gene by PCR. The colistin resistance plasmids are currently being sequenced using an Oxford Nanopore MinION sequencer. The plasmid persistence in pathogenic bacteria is being studied in plasmid stability assays. Eleven MDR-plasmids were identified, including six conferring resistance to colistin. The mcr-1 gene was not identified in any colistin MDR-plasmids by PCR. The analysis of the sequenced plasmids will help to identify ARGs, particularly, novel genes responsible for colistin resistance. The results of plasmid stability assay will identify the ability of MDR-plasmids to persist in new bacterial hosts. The presence of MDR-plasmids in treated wastewater raises awareness about the dissemination of ARG from WWTPs into the environment. Currently no legislative requirements relating to treatment of or concentration of antibiotics, antibiotic resistant bacteria or ARG in treated effluents exist. Conventional WWTPs are not specifically designed to treat or remove these contaminants.

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How Nutrients affects *Coniphora puteana* and *Phallus impudicus* there formation of there cords and how it effects there interaction

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Late-stage wood decay fungi include a specialist group that are able to form hyphal cords which grow out from the colony to forage for nutrient resources across the forest floor. These cord-forming fungi such as *Coniphora puteana* and *Phallus impudicus* are essential for ecosystems due to their ability to forage large distances and access nutrients from decaying wood that would otherwise be inaccessible to other members of the forest floor community. This research investigates how the growth of these two fungi are affected by nutrient availability: how the cord growth is affected by the addition of nutrients, how these fungi interact with each other and how their morphology can change with the addition of extra nutrient sources. *C. puteana* and *P. impudicus* have cords that have very different morphology, and therefore respond differently to nutritional stimuli.

This work was conducted on agar plates with varying richness of nutrient availability, and utilising the addition of agar discs containing extra nutrients to simulate food resources to be searched for. The results showed that cord extension was slowed down by a general increase in nutrient availability and by the addition of extra nutrient resources. The interaction plates show that the faster fungal cords can extend the greater the proportion of available nutrients it will reach and utilise for survival. The data we will present demonstrate the ability of these special fungi to respond to nutrients in the immediate environment and the development of cords to acquire these nutrients, particularly in a competitive setting.

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Growth and activity response of ammonia oxidisers in sediment microcosms under varying salinity concentrations

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Aerobic ammonia oxidation is the rate-limiting step of nitrification and is mediated by ammonia oxidising bacteria and archaea (AOB/AOA). This study focuses on nitrification in Kinvarra bay on the Atlantic coast of Ireland. The specific objectives were to determine and compare nitrification rates and gene abundances across a natural salinity gradient, secondly, to determine autotrophic growth of AOA and AOB across different salinities. Furthermore, to identify actively growing AOA and AOB in coastal sediments using a technique called stable isotope probing (SIP). Using this approach we report for the first time, the autotrophic growth and activity of Nitrosoarchaeum like AOAs in a low salinity habitat originating from intertidal coastal sediments, despite, the numerical dominance of AOB (2.18 x 108 - 6.08 x 108 AOB gene copy numbers g-1 sediment versus 6.22 x 105 - 1.72 x 106 AOA gene copy numbers g-1 sediment). AOA thrived in low salinity environments and could withstand high salinity fluxes for short periods of time. However, AOA amoA gene copy numbers dropped significantly at high salinity (One way ANOVA; $P < 0.001$) and growth of AOA stopped. AOB activity remains ambiguous and further investigation is required to elucidate whether AOB actively nitrify at high salinity. Overall, this study has confirmed the autotrophic growth of AOA in a low salinity habitat, thereby, contributing to our understanding of ammonia oxidiser activity influenced by salinity, an environmental variable found in coastal ecosystems.

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Increasing accuracy of powdery mildews (Ascomycota, Erysiphales) identification using previously untapped DNA regions

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The powdery mildews (Ascomycota, Erysiphales) are a group of obligate biotrophic fungi found on nearly 10,000 angiosperm plant hosts globally including many that are important horticultural and agricultural plants. Infection can greatly reduce the appearance and vigour of the host therefore reducing attractiveness and yields significantly. A reliable and efficient method is required for unambiguous identification of these often cryptic species such that spread to new areas and/or new hosts can be detected rapidly and controlled early. This research aims to combine currently accepted techniques - host identification, fungal morphological analysis, DNA sequencing of the fungal rDNA ITS region - with sequencing of additional nuclear DNA regions in order to increase the reliability of the identification process via BLAST, DNA Barcoding, and phylogenetic reconstruction. Samples were collected through the Powdery Mildew Survey (a citizen science scheme), begun in 2014 and concluding in 2016. Generic fungal DNA primers were found to amplify non-powdery mildew species, some of which were hyperparasites, as well as powdery mildews, and were therefore not a useful technique for accurate identification of powdery mildews. Consequently specific primers were developed for the amplification of the β-tubulin, actin, IGS, chitin synthase, elongation factor-1α, Mcm7, and Tsr1 regions. Results indicate that several of these regions could be used alongside ITS to increase identification power (reliability and accuracy), with particular regions standing out. These rapid diagnostic techniques could provide a valuable tool for plant quarantine, particularly for greater security in the movement of plants and plant products in trade.

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In silico screening of non ribosomal peptides lead to the identification of the biosynthetic pathway of lipopeptides and siderophores

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Microorganisms are considered one of the most important sources of secondary metabolites including ribosomal and non ribosomal peptides (NRPs). The search of new non ribosomal peptides has been motivated by their wide applications exploited by industries in different area including pharmaceutical and phytosanitary sectors. They are produced through complex synthetases called non ribosomal peptides synthetases (NRPS). More than 70 % of NRPs have a complex structure that includes at least one cycle and branches. Therefore, development of specific bioinformatic tools dedicated to the screening of these peptides is necessary as they cannot be predicted and analyzed as classical peptides. With the aim to further analyse the biosynthesis pathways and to identify new active peptides, we have developed a workflow allowing prediction of new secondary metabolites from genomic data using two bacterial models represented by Burkholderia and Aeromonas. Screening of NRPs led us to identify strains probably of interest, regarding their potential of secondary metabolite production. As in the case of Burkholderia, we have detected biosynthetic gene clusters for known products such as cepaciachelin, discover new cluster for new siderophores (phymabactin) and lipopeptides (burkhomycin). It also gave an interesting new insight into the mechanism of nonribosomal synthetases, exemplified by the detection of dual C/E domains in NRPS involved in the production of CLPs by Burkholderia. Analyses of domains organization led to the discovery of a unique mode of synthesis involved in the synthesis of amonabactins, siderophores produced by Aeromonas.

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Screening of *Xanthomonas oryzae* (*Xoo*) phages in rice

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Aim: The aim of this study was to determine the potential phages which possible to control *Xanthomonas oryzae* in rice. As known that rice (*Oryza sativa*), is a staple food for more than 2.7 billion people worldwide. However, it is very susceptible to a number of diseases, among which the bacterial blight disease (BLB). These bacteria can infect rice from seedling stage to mature plant and the disease is manifested by either leaf blight or kresek symptoms.

Methodology: A comprehensive survey of various agro-ecological zones in Malaysia was conducted for infected rice plants at different stage of planting. 100 samples of rice at panicle initiation stage which showing the typical bacterial blight symptoms were collected from 5 granary areas in Peninsular Malaysia. These pathogen and phages were isolated based on basic microbiology methods, biochemical tests and identified using 16S rRNA analysis.

Results: A total of 10 bacteria isolates showed similar character and identified as *Xanthomonas oryzea* based on phylogenetic analysis. For phages, 14 of *Xoo* phages showed some positive inhibition against *Xanthomonas oryzea.* These *Xoo* phages will be further characterized, identified and mass produced for further field trial study.

Conclusion and future work: Those potential *Xoo* phages could be an alternative biocontrol agent for bacterial blight disease. However, more studies need to be carried out, especially on the infection mechanism of *Xoo* phage on this pathogen cell.

Keywords: *Xanthomonas oryzae*, biochemical tests, rice (*Oryza sativa*), bacterial leaf blight (BLB)

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Global distribution and ecology of photosynthetic pigment types of the marine *Synechococcus* picocyanobacteria

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Synechococcus is the second most abundant phytoplanktonic organism of the oceans, significantly contributing to global primary production. This picocyanobacterium displays a wide diversity of photosynthetic pigments, reflecting the variety of light quality niches colonized by this ubiquitous microorganism. Three main pigment types and several subtypes can be defined based on the phycobiliprotein and chromophore content of their light harvesting antenna (phycobilisome), each being specialized into collecting a specific range of the light spectrum. While most strains exhibit fixed pigmentations, some strains are chromatic acclimaters and can tune their absorption properties to optimally collect either green or blue light. Interestingly, pigment content is independent of the core genome phylogeny, and specific approaches are thus needed to study the distribution of pigment types in the environment. Using comparative genomics on physiologically characterized strains, we demonstrated that a combination of 3 genetic markers can be used to predict all known *Synechococcus* pigment (sub)types. We developed a pplacer-based bioinformatic pipeline that accurately assigns sequencing reads to the corresponding pigment (sub)type. The analysis of 137 metagenomic samples from the Tara expedition revealed the distribution of blue and green light specialists as well as chromatic acclimaters, the latter being the most abundant pigment type at depth. Surprisingly, pigment types abundances were correlated not only with optical parameters but also temperature and nutrients, suggesting a complex interplay between vertically inherited traits and pigment content for niche adaptation. Altogether, this study provides unprecedented insights into the adaptation of a major component of the phytoplankton to environmental light niches.

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BACTERIAL PATHOGEN RESPONSIBLE FOR URINARY TRACT INFECTION IN PATIENT ATTENDING TO GENERAL OPD OF B.P.KOIRALA INSTITUTE OF HEALTH SCIENCES, DHARAN

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Introduction: Aim: To identify the micro-organisms causing urinary tract infections and determine antimicrobial susceptibility pattern of the isolated micro-organism responsible for urinary tract infection. Backgroung: Urinary tract infections (UTIs) are the common cause of bacterial infection. Recently UTI become more complicated and difficult to treat because of appearance of pathogen with increasing resistance to antimicrobial agents. It is useful to obtain the local sensitivity pattern in the hospital setting so as to guide empirical prescribing.

Methods: This is a prospective hospital based study to identify the organisms causing UTI and their antibiotic susceptibility. A total of 400 urine samples were collected from the patient presenting with symptoms of UTI. Quantitative urine culture was performed by using calibrated loop direct streaking method of 4 mm. diameter nichrome loop delivering 0.001ml. of uncentrifused urine specimen which was inoculated onto cysteine lactose deoxycolate agar medium and incubated at 37 degree centigrade for 24 hours.

Results: Out of 400 patients, 163 (40.7%) showed bacterial growth. *Escherichia coli* was most common (68.7%).The most effective antibiotic was Nitrofurantoin (89.7%).

Conclusion: The most common causative organism for UTI was Escherichia coli, and the best first line antibiotic was Nitrofurantoin.

This study provides useful information for health practitioners off all levels in the eastern region of Nepal.

Key words: Bacterial pathogen for UTI and first line antibiotic

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Naturally derived lactic acid bacteria as a potential probiotic vaccine against bovine TB in wildlife

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Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB), resulting in significant financial losses for the British cattle industry. The annual cost to the UK government in controlling bTB is ~ £100 million, primarily coming from herd 'test and slaughter' and the ensuing compensation to affected farmers. In parts of the UK the European badger (*Meles meles*) is a reservoir of infection to cattle, making eradication of the disease particularly difficult. The culling of badgers in an attempt to address this transmission route is contentious. Whilst a TB injectable vaccine is available, the need to trap and inject badgers places constraints on its wide-scale adoption. A cheap alternative means, such as oral vaccination, to break the transmission of infection from badgers to cattle would be highly desirable. In this study we isolated lactic acid bacteria (LAB) from badger faeces. The initial observation was that these isolates demonstrated an inhibitory effect against *Mycobacterium bovis* BCG suggesting that these natural gut microbiota could have implications for oral vaccination. Further work was undertaken to exploit these LAB isolates as a probiotic vaccine vehicle to express the *M. bovis* antigens for a potential oral badger vaccine against bTB. To date there have not been any studies described in the literature for badger-derived LAB. These isolates are suitable for this particular purpose given their immuno-stimulatory properties and the fact that they are naturally derived from the host species. This work addresses a novel concept that has potential applications as a platform for recombinant vaccines.

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The temporal and spatial dynamics of tick borne disease in Cumbria

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B. burgdorferi sensu lato and *B. miyamotoi*, are known to be present in the UK. *B. burgdorferi* s.l. is the causative agent of Lyme borreliosis. *B. miyamotoi,* is an emerging pathogen, recently detected in the UK for the first time, but yet to be associated with human disease. *Ixodes ricinus* is the principal vector of both of these spirochetes in the UK.

This project aims to quantify the environmental threat of *B. burgdorferi* s.l. and *B. miyamotoi* and determine the drivers of this threat by long-term monitoring of *I. ricinus* populations in southern Cumbria. Questing *I. ricinus* ticks have been collected every 4 weeks since June 2013 and tested for the presence of *B. burgdorferi* s.l. and *B. miyamotoi* using molecular methods. Results to date have demonstrated the expected seasonal variation in tick density but consistent differences between the density of ticks at each site. The prevalence of *B. burgdorferi* s.l. at each site has fluctuated markedly and there have been consistent differences between infection prevalence at each site. Four *B. burgdorferi* s.l. genospecies have been detected across the sites although the relative contribution that each genospecies makes to the borrelial community at each site has varied markedly. *B. miyamotoi* has been consistently detected in the study area.

B. burgdorferi s.l. infection prevalence in ticks varies spatially and temporally thus reliance on single cross-sectional surveys to estimate local Lyme borreliosis risk could be misleading. More work is needed to understand the ecological determinants of the observed variation.

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HAPIE: A New Tool for Exploring Phenotypic Information from Halophilic Archaea

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Knowledge of extreme halophiles (organisms thriving in salinities >20% NaCl) is ever-growing with much still unknown. They are at the forefront of discovery in biotechnology with applications in bioremediation, biocomputing, bioplastics and possible new antimicrobials.

The most extremely halophilic microbes belong to the archaeal class *Halobacteria*. This taxonomically well-defined and phenotypically diverse group contains approximately 213 species assorted into 57 genera, with their associated data usually being scattered across individual publications. Our aim is to create the first online, publicly accessible database containing phenotypic data on all described species within this group.

Here we present a summary of the ongoing work to establish the Halophilic Archaea Phenotypic Information Explorer (HAPIE), and present our first results. Our project will provide an extensive and complete overview of the capabilities and features of this class and the genera/species within. This tool will be of great use for rapid comparison of large amounts of data, enabling the analysis of trends of taxonomic relevance, as well as for use in research, for the identification of particular strains with relevant capabilities, and to discover possible new applications.

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The modern alchemist and high value biopolymer production from waste.

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Polyhydroxyalkanoates (PHAs) are a group of biocompatible, environmentally neutral, biodegradable plastics that can be produced by certain bacteria. Some of the factors limiting the mass usage of PHAs are the high production costs, due to the carbon sources required, and their expensive chemical processing requirements. This study introduced the novel use of oxidized polyethylene wax (O-PEW) and non-oxidized polyethylene wax (N-PEW) substrates as a carbon source for bacteria. The waxes used were obtained from a process that utilized shredded waste plastics and melted them down into consistent PE waxes. These waxes were then fed to bacteria to make PHAs. The bacterial strain of Cupriavidus necator H16 was selected for the study due to its ability to produce PHAs. It was grown for 48 hours in nitrogen rich or nitrogen-limited conditions that were supplemented with O-PEW or N-PEW. Under these conditions the accumulation of PHAs varied from 20% to 40 % (wt / wt) of dry biomass in both media. The biopolymers produced were analysed using FTIR, NMR, TGA and ESI-MS/MS. Analyses revealed that the PHAs obtained were 3-hydroxybutyrate and up to 3 mol % of 3 - hydroxyvalerate as well as 3 - hydroxyhexanoate co-monomeric units. It can be concluded, that O-PEW and N-PEW could be promising carbon sources for PHA production. We have also demonstrated that the addition of O-PEW and N-PEW to the fermentation medium can have an influence on the structure of the biopolyesters made and variation in wax acid number can affect yield.

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Identification of colistin and imipenem resistance in bacterial isolates from Irish pigs

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The World Health Organisation currently considers antibiotic resistance (AR) one of the greatest threats to animal and human health. It is crucial that all sources of AR are identified and controlled to minimise the transfer of resistance genes and/or bacteria within animals, and between animals and humans. Imipenem and colistin are two antibiotics that play key roles in the treatment of multi-drug resistant bacterial infections. However, resistance to these antibiotics have recently been identified in food animals. The aim of this study was to investigate AR in bacterial isolates from Irish pigs. Selective agars and antibiotics were used to isolate AR bacteria from pig faecal samples, and the cultured isolates underwent antibiotic susceptibility testing. Thus far, this work has identified 16 isolates demonstrating resistance to colistin, seven isolates with resistance to imipenem and five of these isolates exhibit resistance to both antibiotics. Furthermore, 15 isolates displayed reduced susceptibility to imipenem. Our initial results have identified AR in pig faecal samples and highlights the need to determine the mechanisms leading to resistance for these antibiotics in food animals. The emergence of AR in the environment, the use of antibiotics in veterinary medicine and the possible transfer of resistance through the food chain to humans are issues of high priority at both the national and EU policy levels. It is essential that AR in food animals is investigated to ensure the maintenance of animal and human health, and guide future policy.

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The removal of micro-pollutants from wastewater – Harnessing bacteria

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We aim to identify a means to efficiently remove micro-pollutants from wastewater, by utilising bacteria. In order to achieve this we are isolating and identifying micro-pollutant degrading bacteria with the hope of detecting key genes involved in these processes. Currently we have identified degraders of estrogen and intend to use this as our "proof of principal". Although the presence of estrogens in natural water systems is well known, in addition to the ecotoxicological effects, little is known about the degradation pathways. Therefore, we have sequenced the genomes of known estrogen degraders with the intention of isolating the genes responsible for degradation, and also to piece together the steps involved in these pathways. By using a variety of molecular biology techniques, in conjunction with metabolomics, we aim to identify operons associated with estrogen degradation and begin the process of characterising the enzymes involved. Not only will this be beneficial for furthering our goal of utilising bacteria for micro-pollutant removal but it will also increase our general understanding of estrogen degradation. We feel this is an excellent starting point for examining the role bacteria play in removing estrogen from the environment.

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Fitness analysis of tetracycline resistant and sensitive *Shigella flexneri* in lethal and sub-lethal concentrations of tetracycline in Thames river water microcosms.

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It is becoming increasingly important to monitor how antibiotics influence fitness of microbial populations in the environment due to emergence of antibiotic resistance. We analyse the fitness of resistant and sensitive *S. flexneri* strains under tetracycline selection pressures in Thames River river water environments.

Microcosms of Thames water from upstream, central London region and downstream were inoculated with zero, lethal and sub-lethal concentrations of tetracycline were prepared. 10^4 CFU/mL isogenic pair of *Shigella flexneri* 1100 (resistant) and 1363 (sensitive) were added to each microcosms at 1:1 ratio. Fitness was measured by comparing resistant and sensitive colonies after two days of selection pressure. PAH concentration and tetracycline degradation was determined using UPLC with fluorescence detection and HPLC with tandem mass spectrometric detection, respectively.

Interestingly, *S. flexneri* 1363 was found to significantly outcompete the resistant 1100 at sub-lethal and lethal concentrations of tetracycline in downstream microcosms, where PAH levels were highest. Upstream however, *S. flexneri* 1100 outcompeted the sensitive strain in the same environments. The concentration of benzo(a)pyrene in upstream, central London and downstream samples was found to be 0.24µg/mL, 1.35µg/mL and 30.79 µg/mL, respectively. Phenanthrene was detected at 1.10 mg/L, 20.51 mg/L and 140.90 mg/L in upstream, London and downstream sites respectively. Tetracycline degradation was observed in downstream lethal samples from 25 µg/mL to 10 µg/mL over 5 days.

Our results suggest PAHs in the environment may render the tetracycline unavailable. Resistance genes become a selective disadvantage where tetracycline is not bioavailable. Degradation of tetracycline may also contribute to the bio-unavailability.

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A sneak preview of the private life of a pioneer: introducing *Vuilleminia comedens*

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Vuilleminia comedens is one of a small group of saprophytic fungi whose role it is to break down lignocellulose and make the carbon locked up in trees available to the rest of the ecosystem. *V. comedens* is a specialist within this group of wood decay fungi as it is a pioneer species. It is part of the primary decay community which is able to establish itself in dead but undecayed wood. It is out-competed by faster growing secondary fungal colonisers once decay has started, but what *V. comedens* contributes to wood decay and what it produces during those initial steps in the process are unknown. It is known that pioneer species have an impact on subsequent community development, but how this is manifest in wood decay is unknown.

This work studied *V. comedens* as it grew in beech wood. Extraction of high quality RNA from wood is challenging, but here we reveal the assembly of a *de novo* transcriptome of *V. comedens* during wood decay, showing how this unusual fungus claims space and nutrients in its natural habitat. We also studied the extracellular proteome produced during this wood decay process, and will discuss functionality in the context of primary colonisation. The genome of this pioneering species is not yet sequenced, but this project allows a look at what *V. comedens* is doing at a molecular level, and how that may enable establishment of the wood decay community.

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Enhanced visible light inactivation of Clostridium difficile and Norovirus within simulated faecal contamination

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Frequent bowel movements associated with Norovirus and *Clostridium difficile* infection, and shedding the causative microbes in faeces, results in environmental contamination and increased infection transmission. This study demonstrates the efficacy of antimicrobial 405nm light - which has demonstrated success for environmental decontamination - for the enhanced inactivation of Norovirus and *C. difficile* spores when in the presence of faecal matter.
C. difficile spores and feline calicivirus (FCV; used as a norovirus surrogate) suspended in artificial faeces, were exposed to high intensity 405 nm light using irradiances of >150 $mWcm^{-2}$, and the dose response kinetics observed.
Results demonstrate the enhanced sporicidal and viricidal effects of 405nm light against *C. difficile* spores and FCV when in the presence of artificial faecal matter. For FCV, >4log_{10} reduction was achieved when suspended in artificial faeces using 1.4$kJcm^{-2}$: 50% less dose than required for inactivation in PBS. A similar enhancement was observed with *C. difficile*, with a 33% reduction in dose achieving inactivation in faeces compared to PBS (2.4$kJcm^{-2}$).
Analysis of the suspending media showed reduced transmission of 405nm light through the faeces compared to PBS, but organic matter within the artificial faeces may be predisposed to photosensitization, thus enhancing the inactivation process. Overall, results have shown that inactivation of these key organisms can be enhanced when suspended in faecal matter, and this may have positive implications for enhancing decontamination using 405nm light in clinical environments, with potential for use within the bathrooms of patients with gastrointestinal infections alongside current cleaning and infection control measures.

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Gel-stabilized gradient plates: A new approach to microbial strain cultivation and isolation

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The natural habitats of most microbes are extremely dynamic and typically include spatial gradients (of e.g. growth substrates, electron acceptors, pH, salts, and inhibitory compounds). The isolation of new microbial strains from environmental samples should therefore ideally rely on culturing techniques that reflect such conditions. This is not the case, as most cultivation-based studies still rely on the use of standard plating techniques, using homogeneous media and conditions that fail to mimic *in situ* conditions. New tools and approaches are necessary, particularly as the use of inadequate techniques and conditions is consistently pointed as one of the reasons why most of the microbial diversity still eludes our cultivation attempts.

Here we report on the development, optimization, and application of plate diffusion methods to replicate natural salinity and pH gradients. Furthermore, we demonstrate the applicability of this technique by successfully separating microbial strains with different growth requirements along these gradients. This technique represents a new application of gel-stabilized gradient plates, providing a promising new tool for the artificial recreation of naturally occurring interfaces and for microbial strain cultivation and isolation.

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A double blind, randomised, placebo-controlled trial of *Lactobacillus acidophilus* for the treatment of acute watery diarrhoea in Vietnamese children

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Probiotics are the most frequently prescribed treatment for children hospitalised with diarrhoea in Vietnam. We were uncertain of the benefits of probiotics for the treatment of acute watery diarrhoea. We performed a double blind, placebo-controlled randomised trial of children hospitalised with acute watery diarrhoea in in Vietnam. Children meeting the inclusion criteria were randomised to two daily oral doses of 2×10^8 CFU of a local probiotic (*Lactobacillus acidophilus La-14*) or identical placebo for five days as an adjunct to standard-of-care. The primary endpoint was time from the first dose of study medication to the start of the first 24-hour period without diarrhoea. Secondary outcomes included the total duration of diarrhoea and hospitalisation, daily stool frequency, treatment failure, daily faecal concentrations of rotavirus and norovirus, and *Lactobacillus acidophilus* colonisation. 150 children were randomized into each study group. The median time from the first dose of study medication to the start of the first 24-hour diarrhoea free period was 43 hours (inter-quartile range (IQR) 15-66 hours) in the placebo group and 35 hours (IQR 20-68 hours) in the probiotic group (acceleration factor 1·09 (95%CI 0·78-1·51); p=0·62). There was also no evidence that probiotic was efficacious in any pre-defined subgroups or secondary endpoints. No adverse events were recorded. This was a large double blind, placebo-controlled trial in which the probiotic underwent longitudinal quality control checks. *Lactobacillus acidophilus La-14* was not beneficial in treating children with acute watery diarrhoea.

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Survival of *Salmonella* Typhimurium DT104 and *Salmonella* Senftenberg 775W on Blanched Almonds and Skin-On Almonds Exposed to Hot Oil.

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Background: *Salmonella* has been identified as a potential biological hazard in raw tree nuts and as a mechanism of control, processors use various non-thermal and thermal technologies including oil roasting for processing. The effect of hot oil on *Salmonella* Typhimurium DT104 (*S*T-DT104) and *Salmonella* Senftenberg 775W (*S*S-775W) inoculated onto almonds was investigated in this study. **Methods:** Blanched and raw skin-on almonds were artificially inoculated with *S*T-DT104 and *S*S-775W, 50g portion of the inoculated nuts were then exposed to rapeseed oil maintained at 90°C, 100°C, 110°C, 120°C, 130°C, 140°C and 150°C for 1min. The effect of time on the survival of *Salmonella* was also studied at a fixed temperature of 110°C for a maximum of 4mins. The level of *Salmonella* before and after exposure to hot oil was enumerated on selective Bismuth Sulphite Agar and Tryptone Soya Agar. **Results:** Level of *Salmonella* after artificial inoculation ranged from 7.87 ± 0.1 to 8.95 ± 0.2 logCFU/g. After treatment at 150°C level of *S*S-775W on both almond types were below detection limit, however level of *S*T-DT104 were approximately 2 logCFU/g on both almond types. *S*T-DT104 were observed to have a D value of 39.7s for skin-on almonds and 41.8s for blanched almonds at 110°C. *S*S-775W were observed to have a D value of 32.1s for skin-on almonds and 34.5s for blanched almonds at 110°C. **Conclusion:** Heat resistance of *Salmonella* was higher on blanched almonds as compared to skin-on almonds. Also *S*T-DT104 had higher heat resistance than *S*S-775W on both blanched and skin-on almonds.

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Application of photodynamic therapy for bacterial inactivation

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It is an interesting alternative approach to traditional disinfection techniques such as chloronation, ozonolysis and irradiation with UV light because it is low cost, low environmental impact and low maintenance. The process is also a promising ecologically-friendly option for water disinfection because it does not induce bacterial resistance, is not mutagenic or genotoxic. Photosensitisers are frequently based on heterocyclic ring structures such as porphyrins. The aim of this project was to investigate the efficacy of a water soluble singlet oxygen (1O2) generating porphyrin. The porphyrin used was the 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP) in a 200µM stock solution. The experiments were performed in glass Petri dishes (60x15 mm) and irradiated with two different multi-LED lamps, one green at 525nm and one white (400-700nm). Gram positive and Gram negative bacteria were investigated at an initial inoculum size of 105 CFU/ml. The porphyrin concentration used was 3.65µM. Bacterial numbers were monitored using the plate count technique. All samples were analysed in triplicate and dark and positive controls were included. TMPyP showed good inactivation of the bacterial strains. After 180 minutes of light exposure (± 9.7 J/cm2) a 5 log reduction in the numbers of cells was observed for the microorganisms Staphylococcus aureus ATCC 6538, Escherichia coli (wild strain), Escherichia coli ATCC 23716 and Escherichia coli ATCC 25922. A 2 log reduction was observed for Pseudomonas putida ATCC 47054 and Pseudomonas putida DSM 13337. Interestingly, Pseudomonas fluorescens ATCC 13525 responded differently, not showing any deactivation under the same conditions.

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Effectiveness of photodynamic inactivation of microorganisms monitored by culturable and non-culturable techniques

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The interest in finding alternative processes to delivery safe drinking water is growing in recent times. The photodynamic inactivation of microorganisms (PDI) is a promising ecologically-friendly and multi-target approach for water disinfection. It uses visible light as an energy source combined with a photosensitiser (PS) to transfer energy/electrons to a substrate or molecular oxygen, generating reactive oxygen species, which cause cidal effects towards cells. Among the numerous advantages of applying PDI in water treatment are the facts that this technique does not induce bacterial resistance and the low cost, given that sunlight can be the energy source. In this study, culturable and non culturable techniques were used to investigate the response of the bacteria *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538 to PDI, using the porphyrin 5,10,15,20-tetrakis(N-methyl-4-pyridyl)-21H, 23H-porphine (TMPyP). The experiments were performed in glass Petri dishes irradiated with a multi-LED (wavelengths 430nm and 660nm). The initial inoculum size was 10^5 CFU/ml and different concentrations of the PS were tested. The response of the cells to PDI was monitored using the plate count technique and confocal microscopy with ViaGram™ Red. Confocal microscopy also enabled the observation of the porphyrin within the dead cell. Overall, the results were very encouraging with promising applications for water disinfection.

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

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Clostridium autoethanogenum is a strictly anaerobic, Gram-positive bacterium capable of autotrophic growth by fixing carbon via the Wood-Ljungdahl pathway. The organism was first isolated from rabbit faeces and, like other acetogens, produces a variety of fuel-viable solvents when grown on CO and CO2/H2. Analysis of its genome revealed the presence of two putative Agr-type quorum sensing (QS) systems. In other bacteria, these systems are responsible for concerted, population-wide changes in gene expression and behaviour in response to cell population density. The role of QS in C. autoethanogenum is currently unknown but hypothesised to be responsible for sporulation initiation, as shown for other clostridial species. The two systems may also play a role in regulating fermentation metabolism as shown for the related but non-acetogenic C. acetobuytlicum. By using an in-frame gene deletion method, three separate agr mutants were created. These mutants revealed surprising differences from the wild type strain in terms of their fermentation characteristics. An overall increase of ethanol and 2,3-butanediol in the fermentation broth was observed, at the expense of acetate production. Furthermore, head space analysis of cultures grown in serum flask bottles revealed increased CO2 concentrations suggesting reduced CO2 re-assimilation via the Wood-Ljungdahl pathway.

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A single base pair mutation in an integron located D-alanine-D-alanine ligase alters the susceptibility of the host to D- cycloserine

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While investigating integrons (natural gene capture and expression systems) and their associated gene cassettes in the human oral cavity using PCR-based metagenomic approach, we identified two natural variants of D-alanine-D-alanine ligase (ddl) predicted to encode novel variants of the protein. The genes, named as ddl6 and ddl7, were located on the first cassette of an integron and were differed from each other by two nucleotides at c.490 and c.777 in the coding sequence.
We observed that expression of the ddls in E. coli conferred different levels of resistance to D-cylcoserine with an MIC of 16 and 64 µg/mL for ddl6 and ddl7, respectively. To identify which SNP is responsible for this phenotypic change, we used site-directed mutagenesis. The nucleotides at c.490 and c.777 of ddl6 were substituted separately with the corresponding nucleotide of ddl7 by mutagenic PCR primers and the mutated constructs were transformed into E. coli. MIC of D-cycloserine for the E. coli strains harbouring the wild-type and mutated ddls was determined by agar-dilution method. We found that C>T substitution at c.490 on ddl6 did not cause any change in the MIC of D-cycloserine; however, the G>T substitution at c.777 increased the MIC of D-cycloserine from 16 µg/mL to 64 µg/mL, the same as ddl7.
This confirms that the SNP, G↔T at c.777 of the ddls is responsible for changing the D-cycloserine resistance phenotype. The mechanism of how this SNP alters the susceptibility of the host to D- cycloserine is not known yet and will be investigated in future.

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Pioneer life: examining fungal community structure and function in decaying tree branches

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Wood decay is performed by successions of a small, specialist group of fungi capable of decomposing lignocellulose. Decay community establishment begins in the woodland canopy where branches die but remain attached, often for several years before falling to the woodland floor. Formed initially by pioneer species, the community is capable of nutrient capture in the face of significant abiotic stress. Pioneers are so rarely studied in this context that we understand little of their requirements to successfully establish themselves and compete within their community, how they interact with their neighbours and what functions and roles they perform. We do know they are extremely important however, not least because they begin the process of priority effects wherein their identity may facilitate or inhibit successful colonisation by later-arriving species.

We present methods to record the in situ diversity and abundance of the active community present within woody resource units whilst preserving spatial arrangement. Eighty-five species present in 16 attached branches of *Fagus sylvatica* were identified from wood samples using a combination of traditional culture method and amplicon sequencing of the ITS1 region. Communities were characterised by low species richness, diversity and evenness, often being dominated by a single species (33-73% relative abundance). Results of analyses investigating the network of community interactions will be presented. The extra-cellular proteome and metabolome of naturally decomposed wood samples will be described, indicating how pioneer fungi communicate with each other in natural communities, how they mediate these interactions and how conserved these functions are between species.

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Genomic Characterization of Campylobacter jejuni Isolated From Wild Birds in Tokachi area, Hokkaido, Japan

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Poultry is the main reservoir accounting for 80% of human Campylobacteriosis, caused by Campylobacter jejuni. Nevertheless, other contamination pathways exist since C. jejuni can colonize various hosts, including wild birds. However, different C. jejuni lineages tend to colonize specific hosts and vary in outcomes of human infections. Yet, factors prompting the ability of some strains to colonize particular niches and reasons behind its virulence in humans and not in other hosts are unclear.
The library for eight wild birds' isolates was prepared using Nextera and sequenced on a Miseq. Paired-ends reads were assembled, ordered and aligned to reference NCTC11168. Functional annotations were obtained online using RAST. Visual comparisons and alignments were done using BRIG, ACT-Artemis and CLC Genomic Workbench respectively.
The 8 draft genomes were largely syntenic to NCTC11168 used as reference, but have a bigger genome size due to insertions of prophage-like sequences known as C. jejuni integrated elements. Virulence-associated features analysis showed that the flagellar filament proteins genes (flaA and flaB) were truncated and had low sequence homology compared to NCTC11168. Moreover, the ferric enterobactin uptake receptor (cfrA) was either missing or short in the analyzed genomes. The CDT (toxin) operon was found in all genomes. Nevertheless, it was longer of 8 bp and showed high sequence variability. Lastly, exogenous DNA materials such as tetO gene, encoding for resistance to tetracycline, a type VI secretion system and a filamentous hemagglutinin (FHA) protein were identified.
The genomic divergence observed may suggest bacterial adaptation mechanisms necessary for wild bird colonization.

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Investigating Actinomycetes from Scottish Marine Sediments for Novel Bioactive Secondary Metabolites

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The occurrence of antimicrobial resistance among pathogenic bacteria is increasing at an alarming rate and requires novel bioactive compounds to be discovered and developed. Actinomycetes have been the most important source of bioactive compounds, the majority of which have been isolated from terrestrial sources while the marine environment remains relatively understudied. It was hypothesised that Scottish sediment from understudied locations would contain considerable bacterial diversity, and thus an exciting resource for novel bioactive secondary metabolite discovery. This study aimed to isolate and characterise actinomycetes from sediments collected from the West coast of Scotland and to determine their potential to produce bioactive secondary metabolites. Sediment samples were collected and subjected to isolation methods to select for rare actinomycetes. Following taxonomic and phylogenetic classification, the isolates were screened for bioactivity against clinically relevant organisms. The elicitation of secondary metabolites through the application of a variety of fermentation conditions was achieved, this included biological elicitation through bacterial co-cultivation and chemical elicitation. The metabolites from each experimental condition were assessed through liquid chromatography mass spectrometry analysis of the resulting crude fermentation extracts.

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ISOLATION AND VIRTUAL SCREENING OF ANTIMICROBIAL PRODIGIOSIN PIGMENT FROM OXALOTROPHIC *Serratia marcescens* OX_R STRAIN

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Prodigiosin a multifaceted secondary metabolite produced by Serratia spp. having great attention as a promising drug in pharmaceutical concern. Prodigiosin pigment is being extracted from Serratia marcescens OX_R bacterium with oxalotrophic phenotype which was isolated from Indian bat guano sample. Experimentally we found increase in organoleptic characters of pigment by oxalate supplementation in peptone glycerol production media during fermentation. Downstream proceeded as pigment extract prepared in ethanol solvent and bulk amount were separated by silica gel Column chromatography and HPLC technique. Potency of pigment were demonstrated in-vitro as an antibacterial agent against common opportunistic skin surface pathogen Staphylococcus aureus NCIM 5021 strain as killing activity by agar well diffusion method. The virtual analysis includes model generation and Pharmacophore analysis was performed to identify the mode of action and features which are responsible for the antimicrobial activity of the isolated prodigiosin. The docking analysis was carried out to identify the probable mechanism of action of the prodigiosin and was found to be against DNA gyrase protein and that was expressed using the crystal structure of the DNA gyrase. Dyeing property towards cotton and latex polymer was demonstrated by extracted pigment as a natural dyeing agent that may have great potentials in prodigiosin dyed fabrics for nosocomial subjects or burn victim patients.

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Laccase-mediated enzymatic humification of lignin

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Humification, a natural process of organic substance stabilization plays a crucial role in the maintenance of organic matter and carbon sequestration in soils. However, mechanisms on the humification through microbial catalysis were still poorly understood. In the present study lignin composed of polymers with various molecular weights was used as a model to study enzymatic humification. CotA-containing intracellular proteins which showed laccase activities were obtained from *Bacillus subtilis* strain MB42. Besides, recombinant CotA protein derived from MB42 *cotA* gene and expressed in *Escherichia coli* was also prepared. During the incubation period humification degree of the products were monitored through the determination of E4 and E6 values of humic substances. The results demonstrated that ABTS was not only required for laccase to oxidize lignin but served as an inducer for laccase production. When cultivated in spore-forming or lignin-containing medium, higher laccase activities were recorded in intracellular proteins of MB42 than that in nutrient broth medium. The crude proteins or recombinant proteins with higher laccase activities gave lower E4/E6 ratio of the product after reacting with lignin, which demonstrated higher degree of aromatic constituent condensation of humic substances. Formation of P-type humic acid was further confirmed by Δlog K value. Three laccase-containing treatments gave similar pattern in the formation of humic substance, as revealed by the variation of humification index through incubation time and grouping in principal component analysis. This study provided evidence that bacterial laccase involved in the enzymatic catalysis of lignin humification.

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Elucidation of a dearomatising reductase reaction involved in anaerobic degradation of naphthalene

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Naphthalene, the smallest member of the group of polycyclic aromatic hydrocarbons (PAHs), is a frequent environmental contaminant which typically accumulates in anoxic soils and sediments. Here, we report on the identification of key reactions in the anaerobic degradation of naphthalene. Its degradation by sulphate-reducing bacteria is known to proceed via 5,6,7,8-tetrahydro-2-naphthoyl-CoA (THNCoA), which gets reduced to a hexahydro-2-naphthoyl-CoA (HHNCoA) with unknown conformation by an enzyme similar to class I benzoyl-CoA reductases. The downstream pathway was proposed to proceed through β-oxidation-like reactions as indicated by metabolites identified in culture extracts by GC-MS analysis. In previous studies, the THNCoA reductase reaction was measured with NADH as electron donor, but neither could a complete conversion of the added THNCoA be achieved, nor could further metabolites be detected. When analysing analogously performed assays via LC-MS, we could show that small amounts of the product of an HHNCoA hydratase are formed in the assays, but the downstream conversion by an NAD^+-dependant β-hydroxyacyl-CoA dehydrogenase is inhibited by the excess of NADH present in these assays. Experiments with alternative electron donors indicated that 2-oxoglutarate is the natural electron donor of the THNCoA reducing system and low-potential electrons are delivered in the form of reduced ferredoxin via a 2-oxoglutarate:ferredoxin oxidoreductase. With 2-oxoglutarate as electron donor, a complete conversion of THNCoA was observed and further metabolites could be detected. This led us to the proposal of a β-oxidation-like downstream pathway with water addition to HHNCoA and a first ring-fission via a hydrolase acting on a β'-hydroxy-β-oxodecahydro-2-naphthoyl-CoA intermediate.

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What the deep sea can tell us about microwaves

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Background microwaves are ubiquitous in our modern, urban environment. The thermal effects of these electromagnetic fields on biological matter have been well researched. However, possible non-thermal effects remain a controversial subject. Our work utilizes the bioluminescent marine bacterium, *Vibrio fischeri*, as a novel biosensor to probe the effects of low power, pulsed magnetic and electric 2.45 GHz microwave fields. The ultimate aim of this project is to microscopically image these biological effects in real-time using custom-made luminophores, to elucidate the mode of action of microwaves at the molecular level.

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Novel Bio-beads of PVA-Alginate Immobilized Marine Yeasts for the Treatment of Shrimp-Processing Effluent

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Shrimp-processing industries produce effluents containing high amounts of salts, organic matter, and nitrogen. In order to explore a new treatment process for the simultaneous removal of the organic and inorganic load in the shrimp-processing effluent (SPE), a novel system involving marine yeasts immobilized as bio-beads were studied in batch-mode. The marine yeast strains were entrapped in a polyvinyl alcohol-sodium alginate hybrid matrix to stably maintain in the batch-culture setups for SPE remediation. Selected proteolytic and chitinolytic marine yeast strains isolated from Arabian Sea sediments were used for the preparation of the bio-beads. The yeast strains were identified as *Candida* sp. mYJrh9 and *Trichosporon* sp. mYJrh51 by the molecular homology of the 18S-ITS1-5.8S-ITS2-28S rDNA fragment. Bio-treatment of the effluent was performed for a period of 45 days under room temperature. Post treatment, the systems were analyzed for various physicochemical parameter viz., pH, COD, TDS, TAN, nitrate, and nitrite. It was observed that the mixed culture treatment using PVA-alginate bio-beads could remove the initial COD from 1761.21 to 158.5 mg/L along with an 83% reduction in TDS. There was a formation of agglomerates in the treatment flasks, the size of which increased over time and was proportional to the reduction in the suspended solids. The clarity of the SPE post treatment was comparable to that of pure water. The nitrates in the SPE were reduced by 72% after 45 days. The present investigation shows the ability of a novel bio-bead system for the treatment of high organic load effluents such as SPE.

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Molecular Characterization of Multidrug-Resistant *Mycobacterium tuberculosis* isolated from different hospitals in Kathmandu Valley

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Tuberculosis (TB) is one of the deadliest and common major infectious diseases in developing and industrialized countries. Global TB control efforts have been severely hampered by the lack of diagnostic tests that are rapid, accurate, simple to use and can be applied at the point of clinical care. A total of 238 isolates from Kathmandu valley were tested for drug resistance. Extracted DNA was processed for Multiplex Allele Specific Polymerase Chain Reaction (MAS-PCR) for the detection of TB by using MPB64 and IS6110 primers and later mutation in katG and rpoB was detected using specific primers for drug resistance patterns. Out of 238 suspected cases, MAS-PCR was found to be positive for 35 (14.70%) isolates. Among 35 positive isolates, rpoB526 mutation and katG315 mutation was found in 5 (14.29%) and 3 (8.57%) isolates respectively. Two (5.71%) isolates showed resistance to both rpoB and katG confirming the multidrug resistant (MDR) tuberculosis. The use of these assays in the clinical setting would significantly reduce the time to diagnosis of MDR tuberculosis, enabling the administration of appropriate treatment regimens at the outset of therapy and to estimate the economic and disease burden of tuberculosis which is essential to inform health policy, increase disease awareness and assess the impact of tuberculosis control technologies.

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Antagonistic activity of bacterial isolates and *Pleurotus ostreatus* against *Aspergillus flavus* growth and inhibition of aflatoxin production

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Aspergillus flavus induced ear rots and subsequent contamination of maize (*Zea mays* L.) by aflatoxin is a serious food safety issue. As México is one of the countries with maize highest per capita consumption, exposure to aflatoxin represents a serious potential health problem. Use of beneficial organisms such as bacteria and fungi with antagonistic capabilities against *A. flavus* can be applied to minimize aflatoxin contamination of maize. In this work we evaluated the antifungal activity against *A. flavus* and the inhibition of aflatoxin production of three bacterial isolates (*Brevundimonas sp, Chromobacterium sp and Stenotrophomonas sp.*) and one basideomycete fungi strain (*Pleurotus ostreatus*). For the antagonism assays the bacterial and fungal strains were inoculated on PDA medium added with a 10% wheat straw extract 3 cm in distance opposite to pathogenic fungi and incubated at 25 ° C with a photoperiod of 12:12 during 7 and 25 days for bacteria and fungi respectively. The barrier between strains and fungi indicated antagonist interaction between them. Aflatoxin production was detected by fluorescence of agar medium under ultraviolet light. Bacterial strains had an inhibitory effect on *A. flavus* growth after three days and not aflatoxin production was detected. On the other hand for the assays with *Plerurotus ostreatus,* the *A. flavus* growth was slower than the control and fluorescence was detected from day 5 and particularly by contact between both fungi, reaching its maximum at day 11, after that it decreased gradually until day 25 were fluorescence was not detected.

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The effectiveness of organic acids at inhibiting growth and biofilm formation in *Pseudomonas aeruginosa*

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Acetic and citric acid are being used increasingly as topical treatments to control infection in burn wounds. The aim of this study was to determine the effectiveness of seven organic acids on growth and biofilm formation of two strains of *Pseudomonas aeruginosa*, lab strain PA01 and clinical burn-wound isolate PA1054. Both strains were grown in supplemented M9 minimal media plus each organic acid at concentrations from 5mM to 20mM across a decreasing pH range. Growth kinetics were analysed using a mathematical growth model and biofilm production was quantified. There was an organic acid-dependent response, with some organic acids being more effective at inhibiting growth than others. As expected, the effect was pH dependent, with all organic acids becoming more effective at inhibiting both growth and biofilm as the pH drops. Biofilm formation increased as overall growth decreased, until growth was completely inhibited at which point biofilm formation was also inhibited. Propionic acid was shown to have a greater impact on growth, however biofilm production increased compared to the other organic acids. This supports the use of organic acids on burn wounds at quite low concentrations as a topical anti-microbial, but the effects on biofilm formation need to be considered in designing further studies.

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Antagonistic Effects of Foodborne *Vibrio alginolyticus* against *Vibrio mimicus*

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Seafood is commonly contaminated with halophilic vibrios, however, many non-halophilic *Vibrio* species are occasionally cross-contaminated. Interactions among *Vibrio* species in seafood is important as it determines what will become predominant, which will have an impact on the food quality and safety. This study explored such interactions, and presents the discovery of antagonism of foodborne *Vibrio alginolyticus*, a common halophilic seafood vibrio, against non-halophilic *Vibrio mimicus*, an emerging foodborne pathogenic species. It focused on the characteristics of antagonism in a mixed population, the properties of the biochemical substances involved, and the effect of temperatures on the degrees of antagonism. The results from disk diffusion assay revealed that antagonism of *V. alginolyticus* against *V. mimicus* was strain-specific. The inhibitory substance secreted by foodborne *V. alginolyticus* strain by the mid-log phase under batch-culture conditions in different liquid culture media caused a strain of *V. mimicus* to demolish. The inhibition was thought to be the effect of an acid produced by *V. alginolyticus* in the culture media, which lost its activity after being neutralised to pH 7.0 (from an original pH of 5.2–5.5). The antagonistic effect was observed through the disk diffusion assay to be temperature-dependent, which was more clearly exhibited at 25ºC than at 37ºC. Since *V. alginolyticus* is one of the most common seafood vibrios, such an antagonism could potentially occur and could result in erroneous evaluation of food safety and misinterpretation of the responsible microbial agent in foodborne disease investigation.

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An On-going Investigation in to the Ecological Determinants of Lyme Disease in the South Downs National Park, South East England: The potential for 'One Health' Interventions

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Lyme borreliosis (LB) is a tick-borne infectious disease, with UK annual diagnoses trebling over the last two decades. The widening UK distribution of the main LB tick vector (*Ixodes ricinus*) has been linked to deer population expansion. However, the wider ecological determinants that affect the density of infected ticks are poorly understood. Deer have key roles in most, but not all, UK LB disease systems, but they are non-competent hosts for the pathogen itself, and small mammals or birds are usually obligatory disease reservoirs. In addition, the relapsing fever spirochete *Borrelia miyomotoi* was detected in the UK in 2014, but the spread and extent of this emerging human pathogen is still unknown. To date, five sites have been drag-sampled across the South Downs National Park (SDNP); with ticks being successfully obtained from all sites. In addition, ticks have been collected from a further sixteen sites where individual or multiple deer were sampled. Currently, ticks are undergoing genetic analysis to determine the host animals involved in the disease cycle, and the presence of *Borrelia sp*. The study aims to provide a mapped assessment of LB risk across the South Downs National Park, and identify the disease reservoir community composition. The results will help elucidate the causal factors in the SDNP, and support development of policies that avoid or minimise conflicts between public and ecosystem health.

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The use of nano-sized Molecularly Imprinted Polymers for the specific targeting and treatment of bacterial infection of Helicobacter pylori.

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Background: Nano-sized Molecularly Imprinted Polymers (nanoMIPs) specific for the membrane protein Lpp20 of Helicobacter pylori were prepared using solid-phase synthesis. The goal of the project was to make nanoparticles capable of targeting H. pylori with ampicillin. Therefore, the nanoMIPs imprinted by epitope were polymerised in presence of ampicillin and fluorescein acrylate. It is expected that the produced nanoMIPs capable of recognising Helicobacter and targeting it through the delivery of the drug will improve the specificity of drug delivery leading to a patient's benefit, and ultimately, to a positive treatment outcome.
Methods: Synthesis of the nanoMIPs was performed by immobilising Lpp20 peptides to glass beads. Low affinity nanoMIPs and non-reacted monomers were washed away with cold water, whilst the high affinity nanoMIPs were eluted using water heated to 60 □C. The images of nanoMIP binding to the bacteria were generated using confocal microscopy.
Results: A high yield of Lpp20-specific fluorescent nanoMIPs either with or without ampicillin was produced. The binding of Lpp20 - imprinted nanoMIPs to H. pylori was confirmed by confocal microscopy showing binding of single nanoMIPs to bacteria and as well as nanoMIP-dependent aggregation of bacteria.
Conclusion: The use of H. pylori-specific nanoMIPs for the delivery of antibiotics is a novel, cheap and effective way of treating bacterial infections. We envisage that our novel materials capable of providing specific controlled delivery will contribute to a faster clearance of infection as well as to the preservation of a normal microbiota.

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Rubbish to resource: Growing microbes on municipal solid waste

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Population growth, urbanisation and increasing economic prosperity are escalating the rate of municipal solid waste (MSW) production. Globally, the majority of MSW generated ends up in landfills or is incinerated, polluting the environment and contributing substantially to climate change.
On average 40% of MSW is biodegradable and primarily of plant origin. This fraction is an abundant and renewable source of lignocellulose that could be exploited as a feedstock for biorefineries but remains largely unexplored. Our industrial partner Wilson BioChemical has developed an industrial-scale autoclave system that efficiently pre-treats and segregates MSW, generating an organic fibre rich in polysaccharides.
MSW fibre is a unique feedstock due to its extremely variable and heterogeneous composition and contains high levels of contaminating metals and inhibitory compounds. We are screening several fungi and bacteria of both established and potential biotechnological utility to identify a candidate strain that has an intrinsic aptitude for fermenting this complex feedstock. A promising microorganism that can naturally survive on MSW fibre-derived sugars and the associated inhibitors could be developed further through genetic engineering and directed evolution for application in a MSW biorefinery.

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Investigating the susceptibility of laboratory-generated bacterial aerosols to antimicrobial 405 nm light.

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Airborne transmission of infectious organisms is a major concern within the healthcare environment. A number of methods for 'whole room' decontamination, such as antimicrobial 405nm light, are being developed, and it is important that efficacy against airborne, as well as surface-deposited contamination is established. This study demonstrates evidence of the dose-response kinetics of airborne bacterial contamination when exposed to 405nm light.
Aerosols of *Staphylococcus epidermids*, generated using a 6-Jet Collison nebuliser, were introduced into an aerosol chamber designed to maintain prolonged airborne suspension and circulation. Aerosolized bacteria were exposed to increasing doses of 405 nm light, and air samples were extracted from the chamber using a BioSampler liquid impinger, with viability analysed using pour plate culture.
Initial results have demonstrated successful aerosol inactivation, with a 98.4% reduction (1.8 log10 reduction) achieved with 1-hour exposure to low irradiance 405 nm light (P=<0.001). Natural decay of the suspended aerosol was observed, however this was significantly less than achieved with light treatment (P=0.004). Inactivation using ultraviolet (UV) light was also investigated in order to quantify the comparative efficacy of these antimicrobial light regions.
Overall, results have provided early evidence of the susceptibility of bacterial aerosols to antimicrobial 405 nm light. Although less germicidally efficient than UV-light, the benefits of 405 nm light in terms of increased safety for human exposure, provide advantages for a number of applications, including continuous 'whole room' environmental decontamination, where reducing levels of airborne bacteria should contribute to reducing infections arising from airborne contamination.

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Comparison of modified PMA-Quantitative PCR with an established MPN method for the detection of *E. coli* in water samples

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Current guidelines for microbiological water quality are based on conventional culture-based methods (plate counts or most probable number methods) that require at least 18–24 hours for results. The primary objective of the present study was to compare numbers of *E. coli* detected in a wide range of water samples by an industry reference Most Probable Number (MPN) method and an optimized qPCR method for viable detection.

A total of 104 samples were analyzed and *E. coli* quantified by Colilert MPN and a modified PMA-qPCR for detection of viable cells. The relationship between methods was determined by linear regression models and the mathematical basis was provided by ISO 17994:2014.

A strong and significant correlation was observed between methods. According to parameters defined by ISO 17994:2014, the standardized comparison indicate that methods are '*indifferent*' according to a two sided evaluation which, in this case, indicate that "the methods are statistically different but the difference is too small to be of practical significance". From a practical point of view, the methods can be considered to deliver the same results.

Conclusions: The comparison and validation results obtained in this study according to standard reference guidelines indicate the possibility of using our optimized viable detection qPCR as an alternative method for the enumeration of environmental *E. coli* strains in water samples. Therefore, we conclude that PMA-qPCR can be a valuable tool for monitoring microbial water quality parameters and has the potential for commercial application as it is rapid (less than 8 hours) and cost-effective.

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Thermophilic Biogas Upgrading - The Community Behind An Energy Storage Solution

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Anaerobic digestion can convert a huge variety of organic materials (e.g. municipal, food and agricultural waste, energy crops) to high-value biofuels. Additionally, it can be employed to transmute excess electrical supply into fuel, for use when needed.

To further development of a secondary, 'ex-situ' reactor for enriching methane in primary digester biogas, upgrading reactors were established in triplicate and run for 17 weeks. These upgrading reactors were supplied a synthetic mixture of CO_2:4H_2 and a minimal, carbon-restricted medium at three conditions: 55°C, 65°C, and 65°C with re-inoculation. Community compositions for each stage were determined through 16S pyrosequencing of reactor contents to sample the microbial populations involved in this niche application.

The thermophilic, carbon-limited reactors encouraged select populations of *Methanobacteriales* Archaea, the majority of which were outside the resolution of reference sequences at the genus level. Curiously, observed populations were marginal except during times of process disruption. Bacterial populations where overwhelmingly represented by the sparsely characterised OPB54 cluster (Phylum *Firmicutes*), which show strong acetogenic activity. Disruption also encouraged populations of halophilic *Themoplasmatales*, possibly due to stresses involved in sustaining homeostasis.

Strong constraints placed on these upgrading communities select for particular, consistent groups of themophilic microbes. Although methanogens observed in this setup are known to reduce CO_2 with H_2, strong populations of likely acetogens suggest alternative methanogenesis pathways have an important role in supporting niche biotechnology communities.

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Can plant growth promoting rhizobacteria (PGPR) be applied as biocontrol to protect fresh produce from colonisation by human pathogens?

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Increasingly foodborne outbreaks of verotoxigenic *E. coli* and *Salmonella enterica* are being traced to the consumption of fresh produce, such as lettuce and tomato. Strategies are required to reduce the risk of infection from ready to eat (RTE) horticultural products, using natural rather than chemical treatments, to improve food safety. One strategy would be to use PGPR to competitively exclude or inhibit, by secretion of antimicrobial metabolites, colonization of fresh produce by human pathogens.

A number of commercially available PGPR treatments were tested in an *in vitro* competition assay with *E. coli* O157:H7 isolate Sakai. PGPR which were able to out-compete Sakai *in vitro* were taken forward and tested under glasshouse conditions. PGPR were applied to lettuce seedlings at transplant and after seven days. Fourteen days after transplant, lettuce plants were challenged with 10^7 cfu/ml *E. coli* O157:H7 Sakai by root-soak. Colonisation of Sakai on lettuce roots was measured after seven days by viable count on selective media.

Bacillus amyloliquefaciens strain GB03 (Companion®) and *Pseudomonas putida* isolate SCRI_5364 can outcompete *E. coli* O157:H7 Sakai *in vitro*. However, when tested in glasshouse conditions, there is no significant difference in the numbers of *E. coli* O157:H7 strain Sakai recovered from PGPR treated versus untreated lettuce roots.

Application of PGPR, which are effective in competition against *E. coli* O157:H7 Sakai *in vitro*, are not able to reduce colonisation of human pathogens *in planta*. Continued research into pre- and post-harvest controls are required to reduce the risk of foodborne illnesses arising from RTE produce.

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Two T7-like Bacteriophages, K5-2 and K5-4, Each Encodes Two Capsule Depolymerases: Implication in Klebsiella pneumoniae Typing and Treatment

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Background. Capsular types are related to clinical manifestations of Klebsiella pneumoniae infections, including pyogenic liver abscess (PLA) and pneumonia. Capsular type K5 is one of the predominant six capsular types of PLA strains and pneumonia strains.

Methods. Bacteriophages K5-2 and K5-4, which are able to infect and grow on either K30/69, K8 or K5 strains of Klebsiella were isolated and characterized, respectively. Two open reading frames (ORF) encoding putative capsule depolymerases of each phage were identified and their functions were determined.

Results. The first ORF encoding the tail fiber proteins, which have the activity of K30/K69 depolymerase and K8 depolymerase, respectively. The second ORF encoding hypothetical proteins, which almost identical in amino acid sequence and with the K5 depolymerase activity, and allowed these two phages were able to infect K5 strains of Klebsiella. A comparison of the tail gene regions of K5-2 and K5-4 shows that the genes are arranged in a modular or cassette configuration and one specific paired primer was designed for cloning of putative capsule depolymerases from these T7-like phages. Treatment with the K5-4 phage provided significantly increased survival in mice infected with a PLA-associated K. pneumoniae K5 strain.

Conclusion. These results demonstrate two dual host-specific K. pneumoniae K5 phages and their tailspikes exhibit capsule depolymerase activity. Each phage has specificity for capsular types K30/K69 and K5, K8 and K5, respectively, and can be used for the typing and treatment of K. pneumoniae infections.

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THREE METAL-RESISTANT, HYDROCARBON-DEGRADING BACTERIA SPECIES MAINTAINED SELECTIVITY OF METAL BIOSORPTION DURING GROWTH ON SPENT ENGINE OIL IN SINGLE AND TENARY METAL-SUPPLEMENTED SYSTEMS

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This study investigated mechanism of tolerance of soil bacteria to heavy metals while growing on spent engine oil (SEO). Petroleum hydrocarbon contaminated soils were collected from automobile mechanic workshops. The bacterial isolates from the contaminated soils were exposed to 1% and 5% SEO and 10 µg/ml - 1000 µg/ml of heavy metals (Cu2+, Zn2+, Pb2+ and the mixture of the three metals) respectively to determine their hydrocarbonoclastic and heavy metal-tolerance ability. SEO degradation and metal sorption potentials of three selected isolates in growth medium supplemented with Pb2+ (500 µg/ml), Cu2+ (400 µg/ml), Zn2+ (400 µg/ml) and mixed metal salts (Pb2+ - 133 µg/ml, Cu2+ -133 µg/ml, Zn2+ - 133 µg/ml) were assessed weekly for 21 days. Pseudomonas alcaligenes B101, Pseudomonas fluorescens C101 and Stenotrophomonas maltophilia E103 showed highest tolerance to heavy metals and SEO in separate treatments. The presence of metals reduced SEO degradation for all the isolates by factors between 10.7% and 46.7%. SEO utilisation by strain B101 was reduced by 35.1%, 46.7%, 35.1% and 41.7% respectively in the presence of Pb2+, Cu2+, Zn2+ and metal mixture. SEO utilisation by strain E103 was reduced by 10.8%, 43.7%, 43.7% and 30.9% respectively. Conversely, while Pb2+, Cu2+ and the metal mixture reduced SEO degradation by 38.3%, 46.7% and 17.0% respectively for strain C101, the presence of Zn2+ stimulated a 10.7% increase in SEO degradation by the strain. Results from this study showed that these bacterial strains may be exploited in the bioremediation of ecosystems co-contaminated with hydrocarbon and metals.

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Prevalence and diversity of ammonia-oxidising archaea and bacteria in ammonia contaminated groundwater.

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High ammonia concentrations in groundwater can have serious ecological consequences, such as fish kills and algal blooms. Ammonia contamination can be remediated by the biological conversion of ammonium into nitrate (nitrification) and its subsequent reduction into nitrite (denitrification). At a former landfill site with ammonia-contaminated groundwater, in-situ bioremediation was encouraged by manipulating environmental conditions in order to promote the growth of nitrifying and denitrifying microbes. This was accomplished by intercepting the groundwater in a set of permeable reactive barrier (PRB) trenches. The first trench was filled with limestone and aerated in order to promote the growth of the autotrophic nitrifying organisms that convert ammonium to nitrate. The second was filled with mulch and capped to create the conditions needed for heterotrophic, anaerobic denitrifiers. Physico-chemical parameters such as dissolved oxygen and ammonia concentration were measured monthly, and nitrifying and denitrifying bacterial communities were monitored using molecular methods. The community profile and abundance of archaeal and bacterial amoA genes were assessed by terminal restriction fragment length polymorphism (T-RFLP) and real-time PCR, respectively. Dissolved oxygen increased in the nitrifying trench and ammonia concentration decreased along the bioremediation gradient. Concurrent shifts in community structure and abundance of archaeal and bacterial amoA genes were noted. The results suggest that microbial ammonia-oxidisers play a key role in nitrogen cycling in ammonia-contaminated groundwater, and that their diversity and abundance can be influenced by bioremediation interventions.

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Real-time PCR based on novel peptide nucleic acid probe for the detection of methicillin resistant Staphylococcus aureus mecA gene

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Backgroud : Peptide nucleic acid(PNA) is an electrically neutral DNA mimic, in which the sugar-phosphate backbone has been replaced by a peptide of N-(2-aminoethyl)-glycine units linked by the peptide bond. PNA probes hybridize to target DNA with high affinity and sequence selectivity due to electrical neutrality of its chemical structure. This trait enables PNA probes to be more efficient and useful in applying to real time PCR. The purpose of this study was to develop PNA probe based real time PCR for the detection of MRSA mecA gene.

Methods:The primer sequences reported by Murakami et al. were used for detection of mecA gene and were 5'-AAAATCGATGGTAAAGGTTGGC -3' and 5'- AGTTCTGCAGTACCGGATTTGC -3. A PNA probe were designed to target conserved regions in target genes. Real time PCR condition was optimized on the 7500 Fast real-time PCR system(applied biosystems). The primers and probes were evaluated against 54 isolates which were correctly identified by VITEK 2 systems(biomerieux). The limit of detection was determined.

Results : The developed real-time PCR assay accorded with conventional PCR assay for 54 target and non-target strains.The assay was enable to detect 100 gene copies. Moreover, fluorescence probe-based melting point analysis allowed the detection of genetic variation on the target gene region.

Conclusions: The PNA probe based realtime PCR showed promising results for for fast and reliable detection of MRSA mecA gene. The PNA probes would offer advantage of advanced flexibility in probe design.

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Activity of wood degrading enzyme and mycelial growth of *Lentinula edodes* on the liquid spawn culture by oak meal addition

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This study was investigated the effects of liquid spawn of *Lentinula edodes* for application to the farm for mass mushroom fruiting body production. In this experiment, we tried to investigate the mycelia productivity and the activity of enzyme involved in wood degradation according to various liquid spawn cultures of *Lentinula edodes*. The six treatment of liquid spawn cultures were prepared by the addition of various levels of oak meal (850um of particle size) to the soybean liquid culture medium (soybean meal 3g, sugar 30g, KH_2PO_4 0.5g, $MgSO_4$ 0.5g and antifoam 3 mL per liter base) and investigated after inoculation *Lentinula edodes*. The higher level of oak meal addition, the higher mycelial mass after 13 days incubation but the mycelial growth rate was no difference between treatments. All treatments had the carboxymethyl cellulase (CM-cellulase) and laccase activities on solid media. The highest cellulase activity was shown at 3g/L of oak meal treatment while the activity of laccase was the highest in the control (no addition oak meal). Further research is needed to evaluate mushroom fruiting body productivity on the liquid spawn cultures by the addition of oak meal.

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Understanding cyanobacterial growth in legacy nuclear ponds

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There are several legacy ponds containing spent nuclear fuel and other waste products at the Sellafield nuclear site undergoing decommissioning. Some of the ponds are open to the air and can suffer from microbiological blooms during warmer months of the year due to nutrient ingress into the pond. The blooms can significantly reduce visibility, potentially constraining or halting waste retrieval operations from the pond and fouling downstream abatement processes. The cyanobacterium *Pseudanabaena catenata* is a primary coloniser during bloom formation, and a series of experiments were set up to better understand the growth of the organism in low nutrient and high pH conditions characteristic of the pond. The culture was grown in medium designed to mimic pond nutrient levels, and growth was compared to that in standard BG11 medium with reduced nutrients. To assess the effects of pH on cyanobacterial growth, BG11 medium buffered at pH 10, 11, and 12 were also used. The highest cell concentrations that the media can support were determined, in addition to the length of the growth cycle, and the cell growth rate. The data will be used to design flow-through "purging" experiments that will mimick operating procedures in the pond, and evaluate purging regimes as a method of controlling cyanobacterial growth. This research project will provide better insight into the growth of bloom causing microorganisms in nuclear ponds, and assess options for bloom prevention or control.

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Marine microalgae as sources of phospholipids and sterols for use as nutraceuticals and encapsulation systems.

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Background - Addition of nutraceuticals to food products shows promise as diet based prevention for many chronic diseases. Nutraceuticals are often hydrophobic and not easily incorporated into food products. Phospholipids from microalgae are ideal for the production of liposomes, allowing incorporation of the hydrophobic compound of interest, and potentially providing omega-3. Phospholipids have the ability to increase adsorption of DHA in infants, and reduce cholesterol and hepatic fibrosis in mammalian models.
Methods - Three species were studied, (*Cylindrotheca fusiformis* CCAP 1017/2, *Nannochloropsis oceanica* CCAP 849/10, *Isochrysis galbana* CCAP 927/1). Lipid and pigments were extracted from samples taken throughout the growth phase. Percentage total lipid extract was determined and lipid classes were analysed by HPTLC and scanning densitometry.
Results - *C. fusiformis* phospholipids varied over the growth of the culture (3.11-14.51% of total lipid). *N. oceanica* had an overall high proportion of phospholipids, highest at day 5 (21.37% of TL), corresponding to the beginning of logarithmic growth, with the proportion of phospholipids remaining high throughout. Detectable phospholipid in *I. galbana* was only present at day 10 and 13, (1.03-2.79% of TL).
Conclusion - Photoautotrophic microalgae produced without carbon substrates and grown on non-arable land or using water sources unsuitable for terrestrial plants, confer advantages over terrestrial plants and bacteria. Microalgae have potential as future sources of complex polar lipids and phytosterols potentially for use as additives or delivery systems for high value nutraceutical products.

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Effect of Kaffir Lime Essential Oils from Different Extraction Methods on *Alternaria alternata* Growth

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Two extraction methods (water distillation and solvent extraction) of kaffir lime essential oils from their fruit peels, were compared in term of chemical constituents and antifungal activity. The essential oils obtained from both methods were analyzed by GC/MS. Kaffir lime essential oils extracted by water distillation method contained 27% L-limonene, 25% beta-pinene, 17% sabinene, 10% citronellal, 3% beta-citronellol, 1% linalool and other trace compounds. The essential oils extracted by solvent extraction method contained 21% citronellal, 19% beta-citronellol, 10% linalool, 2% limonene, 2% beta-pinene, 1% sabinene and other trace compounds. *In vitro* antifungal activity of kaffir lime essential oils against *Alternaria alternata* were determined by using poisoned food technique. The minimal inhibitory concentration (MIC) of kaffir lime essential oils extracted by water distillation method against *A. alternata* was 0.8% V/V whereas the MIC of the essential oils extracted by solvent extraction method was 0.4% V/V. *In vivo* study using wounded tomato inoculated with a seven millimeter disc of young culture of *A. alternata*, the result showed that the kaffir lime essential oils extracted by either water distillation or solvent extraction methods gave the same MIC which was 1% V/V. Based on these findings, kaffir lime essential oils may be considered as an alternative to chemicals for control of *A. alternata* growth.

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Occurrence, Physiological Studies and Antimicrobial Susceptibility Pattern of *Pseudomonas* species from Ready to Eat Foods in Ibadan, Oyo state, Nigeria.

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This study was conducted to determine the occurrence, physiological properties and antibiotic susceptibility pattern of Pseudomonas species isolated from ready to eat foods in Ibadan. A total of 196 organisms were isolated from wara, smoked shawa (Llisha africana) fish, Meatpie and Kunun zaki. The isolates obtained were identified based on morphological and biochemical parameters as *Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas mendocina, Pseudomonas fragi, Pseudomonas xiamenensis, Pseudomonas alcaligenes* and *Pseudomonas fluorescens*. Results revealed that the highest total aerobic count (TAC) was 2.73x108 $cfug^{-1}$ observed in wara obtained from Bodija market while the Pseudomonas count (PC) was 4.85x107 $cfug^{-1}$ observed also from wara from Sawmill market. The lowest TAC of 1.27x105 $cfug^{-1}$ was observed in smoked fish from Bodija market while the lowest PC of 2.80x103 $cfug^{-1}$ was observed in smoked fish from Moniya market. Physiological studies showed that Pseudomonas species grew best at temperature 30°C (*P.fragi*), NaCl concentration of 2% (*P.putida*) and pH 9 (*P.fragi*). Antibiotic susceptibility showed that 93.5% were resistant to Aztreonam from wara purchased from Oritamerin market. Highest MAR index was recorded as 0.7 (P. xiamenensis) and the least as 0.1 (*P.aeruginosa, P.putida. P. mendocina*). The presence of high load of *Pseudomonas* species in ready to eat foods could be a serious problem to public health especially in the area of antibiotic therapy. In addition, they could serve as reservoir for the spread of antibiotic resistant genes to pathogens resident in the human microflora.

Keywords: ready to eat foods, antibiotics, MAR index, temperature, NaCl, pH.

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Antibacterial activity of repurposed zinc organometallic compounds – from anticancer to effective antimicrobials against E. coli

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Background. Due to the rise in antibiotic resistance there is need for new and effective antimicrobial compounds. The lack in the discovery of novel molecules has turned the attention into drug repurposing being that anticancer drugs have been one main area to explore. In this work we screened the potential antimicrobial activity of three zinc-organometallic compounds (identified as TS263, 265 and 267), previously reported as having anticancer properties.

Methods. The compounds used were tested against E. coli ATCC25922 (reference strain) and non-shiga toxin producing EHEC12900 strain. The determination of the minimum inhibitory and minimum bactericidal concentrations (MIC/MBC) of the compounds, were used to assess their potential antibacterial activity. Toxicity assays were also conducted in normal human fibroblasts in order to determine possible toxicity levels in the human host.

Results. From the three compounds tested, the most active one was TS265 with an MIC value of 4µg/mL and an MBC of 8µg/mL against both E. coli strains. The other two compounds, TS263 and TS267 had an MBC value of 4µg/mL. The highest MIC was obtained for TS263 (16µg/mL). All compounds showed the same MIC values against both E. coli strains. In terms of toxicity, the Co(II) coordination compound CoCl(H2O)(phendione)2][BF4] (phendione = 1,10-phenanthroline-5,6-dione) - TS265, demonstrated lower cytotoxicity towards normal human fibroblasts.

Conclusion. These results are promising and further studies on these compounds will hopefully lead to development of new antimicrobial drugs. Drug repurposing can be an alternative strategy to fight infections caused by antibiotic resistant bacteria.

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Combined therapy to treat antibiotic resistance in clinical isolates of Klebsiella pneumoniae

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Background. Based on scenarios of rising drug resistance, Jim O'Neill and his team estimated on their report, in 2014, that unless action is taken, the burden of deaths from antimicrobial resistance could escalate to 10 million lives each year by 2050. Among these infections are the ones caused by Klebsiella pneumoniae. This is the second leading cause of bloodstream infection in Europe, second only to E. coli. This study focused on the use of combined therapies of antibiotics and adjuvant compounds to reverse antibiotic resistance in multi-drug resistant clinical isolates of Klebsiella pneumoniae.

Methods. A collection of eleven clinical isolates of K. pneumoniae from St. James's Hospital, Ireland was screened for their antibiotic resistance phenotype. This was conducted using the Kirby-Bauer method as per described by NCCLS guidelines. In order to reverse/reduce the antibiotic resistance of the isolates, adjuvant compounds, chlorpromazine (CPZ), thioridazine (TZ), 1-(1-naphthylmethyl)-piperazine (NMP), phenylalanine arginine β- naphthylamide (PAN) were tested in combination with antibiotics. Their MIC values were determined by broth microdilution method and reversals assessed by the checkerboard method.

Results. Eight of the isolates were found to be multidrug resistant. Main resistance was obtained to Trimethoprim, Tetracycline, Cefpodoxime, and Aztreonam. Resistance against Cefpodoxime was particularly relevant as this is a third generation, or 'last resort' cephalosporin. Resistance to TET decreased in the presence of PAN and NMP.

Conclusion. These results indicate that the use of combined therapies can be a good alternative therapeutic strategy to consider in the future for the treatment of K. pneumoniae infections.

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Uncovering the mechanism of action of thioridazine in Salmonella

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Background. The antibiotic resistance crisis is one of the most pressing issues in global health. Thioridazine is a compound that acts against antibiotic resistant bacteria, namely Salmonella, however its mechanism of action has never been fully described. The main aim of this study was to uncover the mechanism of action of TZ in Salmonella enterica serovar Typhimurium 14028S.

Methods. The MIC of TZ was initially determined by broth microdilution. Growth kinetics of S. Typhimurium in the presence of several concentrations of TZ was assessed. The effect of exposing S. Typhimurium to sub-MIC concentrations of TZ was studied by conducting membrane permeability assays (H33342 accumulation) and motility tests.

Results. TZ had an MIC > 200 mg/L against Salmonella. When permeability assays were conducted, S. Typhimurium treated with 200 mg/L of TZ exhibited an apparent loss of cell integrity after 30 mins of exposure to TZ. In the absence of the compound there was little accumulation of H33342, while a concentration of 50 mg/L of TZ rapidly promoted accumulation (increased membrane permeability). Decreased bacterial motility was obtained when the bacteria was incubated in the presence of ¼ and ½ MIC of TZ.

Conclusion. The results obtained suggest that TZ acts by targeting the bacterial cell-envelope. When exposed to TZ cellular permeability increases, resulting in the loss of cell integrity. Findings that S. Typhimurium responds to the in vitro presence of TZ by decreasing its motility may be related with the activation of genes involved in drug resistance and envelope stress responses.

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Detection of Biofilm Production and Antibiotic Resistance Pattern of Staphylococcus epidermidis Isolated from Catheter-Related Bloodstream Infections

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Most Staphylococcus epidermidis-related infections are acquired in hospitals and are associated with the use of medical devices. The aim of this study was to detect biofilm producing S. epidermidis from patients with catheter-related bloodstream infections (CRBSI) and determine their antibiotic resistance pattern.

Methods: Blood and catheter tip specimens were collected from 150 patients with CRBSI. Isolated S. epidermidis strains were tested for biofilm production by the modified tissue culture plate (MTCP) method, Congo red agar (CRA) method, and icaA and icaD genes detection by PCR. Antibiotic resistance pattern of both biofilm producing and non-biofilm producing isolates was tested using the disc diffusion method.

Results: The prevalence of S. epidermidis in CRBSIs was 13.3%. Biofilm production was detected in 55% and 45% of isolates by the MTCP method and CRA method respectively. IcaA and icaD genes were detected in 20% and 30% of the isolates respectively. The sensitivities of CRA method, icaA and icaD were 81.8%, 36.4%, and 54.5% respectively. All of them showed specificity 100%. Biofilm producing isolates were more resistant than non-biofilm producing isolates to cefotaxime erythromycin, rifampin, tetracycline, gentamycin, ciprofloxacin and ampicillin. All the isolates were sensitive to linezolid and vancomycin.

Conclusion: The MTCP remains the best method for screening of biofilm production. IcaD gene detection is more sensitive for detection of biofilm production in S. epidermidis than icaA gene, however, their presence does not always correlate with phenotypic biofilm production. Biofilm producing S. epidermidis isolates showed antibiotics resistance than non-biofilm producing isolates.

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Dual properties of a novel peptide MFAP9 from marine derived fungus *Aspergillus fumigatus* BTMF9: Antibiofilm and anticancer potential.

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Antimicrobial peptides have merited renewed interest due to their potential to disperse microbial biofilms and cancer therapeutic properties. Inhibition strategies based on the planktonic bacterial physiology have been known to have restricted efficacy on the control of biofilm communities. This problem can be intensified by the emergence of increasingly resistant pathogenic strains. In the present study, we have isolated and characterized an extracellular antibacterial peptide designated as MFAP9 from a marine derived fungus *Aspergillus fumigatus* BTMF9 using DEAE Sepharose-based column chromatography. MFAP9 showed a single band of 3 kDa on SDS-PAGE and the homogeneity showed retention time of 32.5 min in RP-HPLC. *In vitro* antibiofilm and antiproliferative properties of MFAP9 were tested which exhibited superlative inhibition against Gram-positive bacteria and lung carcinoma cell line A549. SEM analysis of the bacterial biofilm inhibition after MFAP9 treatment showed complete disruption of biofilm with rupture of individual cells as well. *In vitro* cancer cell viability upon treatment with the peptide fractions assessed by MTT assay and AO/EtBr staining proved significant cytotoxic effects on A549 cell line (IC_{50} - 29µg/mL). This is the first report on isolation of a peptide from marine derived *Aspergillus fumigatus* with a promising potential as an effective tool in medical and therapeutic applications.

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Multi-drug resistant *Vibrio* species isolated from irrigation water and selected vegetables in Amathole Municipalities in Eastern Cape, South Africa

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Antibiotic resistance in pathogenic vibrios is of global public health concern; resulting in difficult to treat infection. In the present study, multiple antimicrobial resistances (MAR) profile of *Vibrio* sp., isolated from vegetables and irrigation water were assessed. Standard culture-based method was adopted for isolating and enumeration of vibrios, while polymerase chain reaction technique was used for confirmation of presumptive *Vibrio* spp. Antibiotic resistance profile of the isolates were tested against eight different classes of antimicrobials. The results showed high levels of resistance against Penicillin G 248/314 (79%) across Amathole district, nalidixic acid 29/35(83%) and cefotaxime 23/35(66%) of *Vibrio* spp. isolated from Ndantsane and Middledrift, as well as chloramphenicol 20/37(54%) and tetracycline 28/37(76%) of the isolates from Konga and Stuttehiem. The results of multiple antimicrobial resistances (MAR) phenotypes for *Vibrio* spp. isolated from irrigation water indicated that 82/150(55%) were resistant to more than two antibiotics while 93/166(56%) were recorded against isolates from vegetables. The MAR index recorded against all *Vibrio* isolates ranged from 0.17 to 0.66. Fifty-five percent 55% (96/175) of isolates that are resistant to more than two antibiotics have MAR indexes above 0.2. These findings revealed that the occurrence of antimicrobial resistance of *Vibrio* spp. were significantly high suggesting that the irrigation water and vegetable samples are potential reservoirs of antibiotics resistances. Thus there is a need for proper surveillance and monitoring of antimicrobial resistance determinants of vibrios in irrigation water and other possible contaminant pathway to the vegetables.

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A study of antibacterial production compound by fungi isolated from petroleum contaminated soil

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The study was conducted during 2015-2016, the soil sample collected from shewashok contaminated soil to test the antibacterial activity of the fungi, 6 soil sample from shewashok petroleum refinery was collected and siol dilution method used to inoculate 30 plate contain PDA, three fungal isolate Penicillium sp, cladosporium sp and aurobasidium sp were obtained and Acetyle acetate and ethanol solvent used to extract antibacterial compounds, the three fungi tested on the three bacteria strain for antibacterial activity and produce zone of inhibition around the bacteria and measuring the diameter of inhibition zone, the penicillum sp extracted compound produce zone of inhibition (13mm) in diameter around E.coli and produce zone inhibition (9mm) diameter around staphylococcus sp and then was no active against pseudomonas sp. and also cladosprium sp extracted compound produce zone of inhibition (16mm) in diameter around E.coli and also inhibit (13mm) in diameter against staphylococcus sp and inhibit (8mm) in diameter against pseudomonas sp. the aurobasidium sp extracted compound produce inhibition zone of (16mm) in diameter around E.coli and inhibit (13mm) in diameter around staphylococcus sp and also inhibit (8mm) in diameter against pseudomonas sp. the result showed that the soil fungi have antibacterial activity against the bacteria strains.

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Effect of chaotropic magnesium chloride on the growth of microbes

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Chaotropic agents denature biological macromolecules and limit microbial growth. Magnesium chloride ($MgCl_2$) is one of the most widespread solutes that exerts chaotropic effects above a certain concentration. We compared the capacity of microbes to grow in a simple medium supplemented with 1% yeast extract and different concentrations of chaotropic $MgCl_2$ and kosmotropic sodium chloride (NaCl), using samples from a salt marsh and agricultural soil (Colchester, UK) and also including a sample from Discovery deep-sea brine interface.

The highest concentration at which salt marsh and agricultural soil microbiota grew was 1.5 M $MgCl_2$. DGGE analysis of these enrichments revealed high community diversity for Bacteria and low diversity for Archaea and fungi. Several bacterial strains were isolated with the capacity to grow up to 1.1 M $MgCl_2$, but fungal isolates proved to be the most tolerant. For example, a *Penicillium* strain from the Discovery brine interface grew up to 1.7 M $MgCl_2$ and a strain related to *Cladosporium* from agricultural soil grew up to 2 M $MgCl_2$.

The nature of the adaptation mechanisms to chaotropic $MgCl_2$ is being investigated further.

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Characterization of CTX-M-14 and CTX-M-15 β-lactamase by in vitro DNA shuffling

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Recently, dissemination of several hybrids of the CTX-M-1 and -9 groups have been identified in Enterobacteriaceae. They were likely formed by recombination between CTX-M-14 and CTX-M-15, the two most dominant variants worldwide. We tried to investigate the evolution of hybrids by in vitro DNA shuffling and identify certain important residues contributed to activity by analysis of shuffled mutants. DNA shuffling is a tool to generate libraries of evolved enzymes by recombination of different genes in PCR. A total of 51 mutants were generated, including 21 hybrids, 30 mutants having backbone of CTX-M-15/-14 with point mutations. The hybrids have various combination while remained active against cefotaxime. The evolution of hybrids from CTX-M-14 and 15 was demonstrated. Certain mutants have CTX-M-14/-15 backbone with few mutations exhibited dramatic reduction in MIC. Mutants with less than 4 point mutations were further analyzed by mutagenesis to identified the critical residues. Mutation of L33, E37, L44, Y60, L119, F151, L199 and L206 in CTX-M-15 exhibited dramatic reduction in MIC to cefotaxime. They were suspected to maintain the stability, indirectly contributing on catalytic activity since they locate far away from active site. Data of thermal stability assay of the mutants showed reduction in melting temperature, supporting their role in enzyme stability. People mainly focus on the active site residues while the key residues identified in this study have not been characterized before. We provide an insight into the residues distal to the active site, which may be potential targets on developing the inhibitors against the extended-spectrum β-lactamases.

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Investigating the effectiveness of anaerobic digestion methods at reducing the prevalence of antibiotic resistant bacteria in waste water treatment processes

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Wastewater treatment plants (WWTPs) can bring together large amounts of waste from different environments with the potential to accumulate vast amounts of bacteria, many of which could carry antibiotic resistance. The solid waste is often treated by anaerobic digestion (AD) and used as fertiliser. Little is known, however, about how effective AD is at eliminating antibiotic resistant bacteria (ARBs) and their genes (ARGs). This work investigates how effective different AD methods are at reducing ARBs and ARGS in WWTPs.

Sludge samples were taken from two WWTPs before and after AD, and also prior to the thermal hydrolysis pre-treatment step carried out in WWTP1, then plated on different types of selective media to enumerate bacteria that survived each treatment step. A selection of colonies were taken forward for antibiotic resistance testing.

Preliminary results show the number of putative *Enterococcus* bacteria was reduced by more than 99.99% after thermal hydrolysis pretreatment, however, approx. 30,000 cfus per ml still survive. *Escherichia coli* appear to be eliminated entirely by the thermal hydrolysis step, however AD without pre-treatment only slightly reduces the numbers of *E. coli*. Preliminary antibiotic testing undertaken on presumptive *Enterococcus* colonies showed that erythromycin resistant bacteria appear to be eliminated after AD, but vancomycin resistant bacteria can be found in AD influent and effluent.

This work will help inform the water industry of the best methods to prevent environmental spread of antibiotic resistant bacteria and genes. Little is known about the impact of using sludge containing ARBs and ARGs as fertiliser.

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Effect of the crude oil on light attenuation and microbial community composition

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Oil spills spread over the surface of the seawater causing the attenuation of light passing through it. We showed that crude oil preferentially attenuates the shorter wavelengths of photosynthetically active radiation. Therefore, we proposed that this change in the spectrum and reduction in light intensity would affect microbial phototrophic community composition, which in turn would alter the heterotrophic community and potentially the rate of degradation. Experiments were conducted using natural seawater with a crude-oil layer above (but not in contact with) the seawater, and a no-oil control, under LED lights. Variations included using continuous light and light-dark regimes, with and without added nutrients. In some experiments, lights were tuned to mimic the effect of oil on the spectrum of transmitted light. Chlorophyll *a* and DNA concentrations were measured as a proxy for biomass. DGGE analysis of bacterial 16S rRNA genes was also performed. Samples under the oil layer had significantly higher concentrations of chlorophyll *a* at the end of the experiments. Nutrient limitation had no significant influence in the concentration of DNA, but chlorophyll *a* concentration was lower compared to those experiments with added nutrients. Samples, under the oil layer, or subjected to the oil-mimicking light spectrum, had greater variability in their bacterial community composition at the end of the experiments than the no-oil controls. Thus, we have shown that, in addition to oil having a direct impact on the microbial community in seawater, it has an indirect effect by altering the spectrum and intensity of light.

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High throughput screening of novel antimicrobial compounds in TruLarv™ *Galleria mellonella*

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The advent of the era of antibiotics was hailed as the end to infectious disease, but over use of these drugs, combined with the ability of bacteria to evolve, means that we are in danger of returning to pre-antibiotic days, in which many infections are untreatable. Across the world, new drugs are urgently being sought. One approach is to identify naturally occurring compounds produced by microorganisms. Historically the Actinobacteria have been a rich source of antimicrobials, but there are many species yet to be tested. In this study several thousand crude actinomycete extracts, were screened for antimicrobial activity, and approximately fifty lead extracts identified. The challenge we faced was how to further screen these extracts for efficacy and toxicity in an infection model. *Galleria mellonella* (Greater Wax Moth) larvae can be used as an inexpensive, ethical and simple to use whole organism infection model. Previously reported studies have shown that there is good correlation between the results of *G. mellonella* infection experiments and existing drug efficacy data in humans. Extracts were tested for efficacy and toxicity in, research grade *G. mellonella* (TruLarv™) larvae in a high-throughput assay. We were therefore able to identify extracts containing bioactive compounds prior to their subsequent purification and testing in mammalian models.

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Survival of Listeria monocytogenes in Soil: Effect of Strain and Soil Type

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Listeria monocytogenes is a deadly intracellular pathogen and ubiquitous in the environment. Ready-to-eat (RTE) foods have been implicated as the source of infection in numerous outbreaks and a recent trend for 'healthy convenience' in the horticultural industry has seen a rise in consumption of RTE fresh leafy produce. Due to significant consumer risk, horticultural companies are subject to increasing pressures to control the level of L. monocytogenes contamination on their product and research is urgently needed to understand how L. monocytogenes contaminates RTE foods. Soil provides an ecological niche for the pathogen and is therefore a potential contamination source but little is known about its persistence in different horticultural soils used to grow RTE leafy produce. Additionally, previous research has failed to recognise the variety of L. monocytogenes strains found in the horticultural environment. Evidence based on commonly used laboratory strains may not be representative of L. monocytogenes strains that are typically found in the growing environment. We are currently investigating the influence of soil texture and strain type on survival of L. monocytogenes in soil microcosms. L. monocytogenes strains used in this study have been previously distinguished by isolation location, sequence type (ST) and growth curve characteristics. Initial results suggest strains isolated from RTE product and the processing environment survive in soils at higher number, for longer than a laboratory reference strain (EGD-e). Furthermore, MLST has revealed a large variety of STs in the UK horticultural industry - these strains have different growth characteristics when grown in culture.

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Wrinkly Spreaders and Goldilocks in Experimental Microcosms

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Adaptive radiation is a key process leading to the development of new species, and is as relevant to the diversification of bacteria as it is for plants and animals. This requires organismal evolvability, ecological opportunity and diversifying selection. In the case of model *Pseudomonas fluorescens* SBW25 populations developing in static microcosms, the metabolic activity of the wild-type colonists generates an O_2 gradient dividing the liquid column into high and low-O_2 zones. This produces the ecological opportunity or niche space for the evolving Wrinkly Spreaders which colonise the air-liquid (A-L) interface by biofilm-formation. Here we investigate how environmental physicality impacts on the fitness advantage Wrinkly Spreaders enjoy over their non-biofilm-forming ancestor. Although O_2 levels were known to be the main driver for diversification and WS fitness in nutrient-rich microcosms, oil-overlay and reduced-nutrient experiments suggest that O_2 flux to the A-L interface and the relative importance of O_2 to other growth-limiting factors is important. Furthermore, manipulation of the shape of the A-L interface impacting on the O_2-rich meniscus trap where cells attach to the vial walls shows that this physical aspect of the experimental microcosm is also important for WS fitness and biofilm-formation. These findings suggests that the Wrinkly Spreader is well-adapted to the conditions found in static microcosms, which is not surprising as ecological opportunity and evolved niche space are considered interlinked in adaptive radiation, in a manner similar to Goldilock's good fortune when she went for a walk in the forest and found the bear's house.

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Neuroprotective activity from crude extract of Microbispora isolated from Thai medicinal plants

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Eighteen actinomycete strains were isolated from the tissue of Thai medicinal plants. These strains were tentatively identified into the genus-level using morphological characteristic and 16S rRNA gene sequence-based phylogenetic analyses. The results revealed that all strains belong to the member of the genus Microbispora. In this study, we fortunately found the strain CL1-1 and KK1-11 showing morphological and chemotaxonomic characteristics typical of members of the genus Microbispora but which was genotypically and phenotypically distinguishable from all recognized Microbispora species. Thus, these strains should be judged as novel species of the genus Microbispora. The fermentation broths of the selected Microbispora strains, CL1-1, CL2-2, KE1-3 and KM1-2 were extracted with ethyl acetate (EtOAc). The crude ethyl acetate extracts were tested for the capacity to produce compounds which can protect P19 neurons from oxidative stress condition. The results revealed that the crude extract at concentration of 1 ng/ml of all four isolates could protect P19 neurons from oxidative stress reached to 87.58%, 75.09%, 79.31% and 63.99%, respectively.

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Staphylococcus Aureus Biofilm Specific Affimers as a Potential Diagnostic Tool for Infectious Endocarditis

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Abstract

Staphylococcus aureus is an important cause of infective endocarditis – infection of the lining of the heart or devices inside the heart such as prosthetic valves/pacemakers. These infections can be debilitating, even life-threatening, and are often difficult to diagnose clinically. *S. aureus* grows on the surfaces of medical devices and natural tissues as a biofilm. Bacteria in biofilms can be difficult to eradicate as they are less susceptible to antibiotics. Thus, biofilms associated with medical devices or chronic infections often require surgical removal or debridement, respectively. Thus, it would be clinically advantageous to be able to not only detect the presence of bacteria when a patient presents with clear signs of infection, but also to determine the source by a non-invasive method. This poster describes the raising of Affimers (a class of non-antibody binding proteins that can be engineered for a wide range of applications where antibodies have limitations) against biofilms and specific extracellular components of *S. aureus*. Biofilm formation was investigated for *S.aureus* strains SH1000, UAMS-1 and USA300. Affimers specific for *S. aureus* strains SH1000, USA300 and UAMS-1 biofilms were successfully generated using phage display and binding specificity was determined by ELISA. Eleven Affimers were tested for *S. aureus* biofilm binding, nine Affimers showed binding specificity to *S.aureus* biofilms. The ongoing characterisation of these Affimers with regard to the breadth of their specificity and their future engineering to allow sites of *S.aureus* infection in patients to be detected will be described.

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The impact of hormones on Pseudomonas aeruginosa urinary tract infections

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Background: Urinary tract infections (UTIs) are associated with significant morbidity, mortality and economic burden. Pseudomonas aeruginosa can cause complicated UTI infections and catheter-associated infections. P. aeruginosa form biofilms which can lead to persistent infections and multi-drug resistance. Evidence suggests that sex hormones may affect P. aeruginosa and that estrogens modulate biofilms and can induce mucoid conversion. Therefore, this study investigates the role of hormones in UTI pathogenesis.

Methods: To study the effect of estradiol, testosterone and progesterone on biofilm architecture, 15 UTI P. aeruginosa isolates were cultured statically for 48 hours in the presence of either 10nM testosterone, estradiol or progesterone. Biofilms were stained using the Baclight® kit and subsequently imaged by confocal microscopy. Upon producing 3D Images by Imaris, measurement of biofilm structure was performed using Comstat. Gene expression was also investigated in 10 P. aeruginosa UTI isolates using qPCR after exposure to 10nM estradiol and testosterone.

Results: 3D images of biofilm structure and subsequent analysis indicate that the effects of hormones on P. aeruginosa are strain and hormone dependent. Similar results were observed for gene expression of virulence genes involved in biofilm formation, quorum sensing and pathogenicity.

Conclusion: Hormones may affect persistence of P. aeruginosa UTI infections by modifying biofilm architecture and altering expression of virulence associated genes. Variation between isolates was observed and could lead to greater recurrence and persistence of UTI infections in some patients. Thus, a greater understanding of gender dependent host-pathogen interactions is needed and may lead to a personalized medical approach.

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Mutants versus cations: variable effects on biofilm formation by *Pseudomonas aeruginosa* PA14

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Pseudomonas aeruginosa is an on-going health hazard for hospitals and water systems, as formation of biofilms makes its eradication rather difficult. In this study, the effect of inorganic salts (10 mM final concentration) on biofilm formation by *P. aeruginosa* PA14 on plastic surfaces was examined, daily up to 96 h. The addition of lithium, manganese and calcium ions significantly speeded up biofilm formation by PA14, whereas the addition of magnesium had no effect. Addition of cobalt chloride appeared to be toxic to *P. aeruginosa* and little to no growth was observed. Furthermore, knockout mutants of five biofilm-related PA14 genes were then tested as above, namely *cupB5*, PA3357, *vfr*, *rh1A* and *ampR*. For the PA3357, *vfr*, *rh1A* and *crc* mutants, the formation of biofilms without the addition of salts was lower than the wild type. There was no change in biofilm formation in the *cupB5* mutant and a substantial increase in biofilm formation in *ampR* was observed. Biofilm formation by the *vfr* mutant was significantly increased by addition of calcium ions. Lithium ions had the same effect for the *rh1A* mutant, while biofilm formation by the PA3357 mutant was enhanced by addition of lithium, magnesium, manganese and calcium ions. Conversely, the addition of any cation in the *ampR* knockout appears to inhibit biofilm formation. Further research is necessary before elucidating the molecular mechanisms behind these effects, however our results suggest that biofilm formation by *P. aeruginosa* PA14 is selectively regulated by specific cations rather than simply by changes in surface charge.

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Impact of *Staphylococcus aureus* on *Candida albicans* biofilm heterogeneity

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Background: Previously, we have shown biofilm formation by *Candida albicans* is highly variable and is a significant clinical entity associated with disease outcome. However, the impact of concomitant bacterial colonisation such as *Staphylococcus aureus* on *C. albicans* biofilm formation remains unclear. We therefore aimed to assess the role of previously identified *C. albicans* biofilm biomarker *AAT1* within dual-species biofilms.

Materials and Methods: Pre-characterised isolates of *C.albicans* with high (HBF[n=5]) and low (LBF[n=5]) biofilm forming ability and *S. aureus* Newman strain were used throughout this study. Mono and dual-species biofilms were grown at 37^{o}C for 24 hours in RPMI. Biofilm biomass was quantified using crystal violet assay and compositionally analysed using qPCR. Scanning electron microscopy was used for biofilm visualization. An *in vivo Galleria mellonella* model was used to assess pathogenicity of mono and co-infections. Aminoasparate transferase (*AAT1*) activity was assessed by transcriptionally and biochemically using a commercially available kit.

Results: We found *S. aureus* significantly enhanced the biofilm formation by LBF (two-fold [$p<0.01$]). Conversely, no change was found with HBF. Microscopic analysis revealed increased biomass of LBF when grown with *S. aureus*. Co-infection of both LBF and HBF with *S. aureus in vivo* demonstrated increased pathogenicity compared to mono-species infection. Analysis of AAT1 activity was shown to be increased within dual-species biofilms of LBF and *S. aureus*.

Conclusions: Collectively this data highlights that *S. aureus* is able to stimulate *C. albicans* biofilm formation potentially via modulating amino acid metabolism that corresponds with increased pathogenicity *in vivo.*

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A study of the inhibition of biofilm formation on multiple surfaces by a biosurfactant containing four discrete lipopeptides

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Microbial biofilms are able to develop on many different types of surfaces including medical apparatus (e.g. urinary and endotracheal devices), implants and food preparation surfaces; there is a need to identify an effective and sustainable means to combat and inhibit biofilm formation. Biosurfactants are relatively nontoxic, surface-active compounds synthesized by a wide variety of microorganisms. They are molecules that have both hydrophobic and hydrophilic domains and are capable of lowering the surface tension and the interfacial tension of the growth medium. They have anti-adhesion properties that can act against biofilm of pathogenic microorganisms.
In this study, eight *Bacillus* strains isolated from farmyard waste were identified using PCR and MALDI-TOF-MS Biotyping system and their capability to produce lipopeptides (a class of biosurfactant) examined. Both methods identified the isolates to species level and are in 100% agreement. The isolates were screened for their ability to produce biosurfactant that inhibits biofilm formation on stainless steel and high-density polyethylene tiles. Biosurfactant from the most promising isolate (a *Bacillus subtilis*) was selected for further study. The biosurfactant was purified using HPLC-RP and characterised by MALDI-TOF and MALDI-TOF-TOF. It yielded four distinct lipopeptides similar to surfactins [m/z 1016-1074], fengycins [m/z 1447-1491] subtilomycin [m/z 3230] and subtilosin-A [m/z 3400]. The crude biosurfactant from this isolate demonstrated anti-biofilm properties against a range of bacteria including MRSA. In most cases, the crude biosurfactant performed as efficiently as a commercially-available purified biosurfactant (surfactin) and was more effective against *Enterococcus faecalis*, *Enterobacter aerogenes* and MRSA than surfactin.

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Fungal and bacterial secreted factors regulate mucormycete germination

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Within the human body, microorganisms reside as part of a complex and varied ecosystem, where they rarely exist in isolation. Therefore, bacteria and fungi have co-evolved to develop elaborate and intricate relationships, utilising both physical and chemical communication mechanisms. Mucormycetes are spore-forming fungi belonging to the order Mucorales and are the causative agent of potentially fatal mucormycosis in immunocompromised individuals. Current therapeutic interventions involving surgical removal of infected tissues and antifungal treatment are often ineffective, with almost 100% mortality in disseminated mucormycosis. Key to the pathogenesis of mucormycetes is the ability to swell and germinate leading to penetration of the surrounding tissues, angioinvasion, vessel thrombosis, and tissue necrosis. These spores are found ubiquitously and most likely encounter a myriad of bacterial and fungal species. For example, mucormycetes have been co-isolated with other trauma- and burn- associated pathogens, such as *Candida albicans* and *Pseudomonas aeruginosa* as well as pulmonary- associated microorganisms such as *Klebsiella pneumoniae*. This study demonstrates that exposure of *Rhizopus microsporus* spores to other pathogenic microbes can inhibit germination. Therefore, it is possible that a predisposing bacterial or fungal infection is able to regulate germination and influence overall fungal pathogenicity through secreted factors.

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Single gene locus changes perturb complex microbial communities as much as apex predator loss

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Many bacterial species are highly social, adaptively shaping their local environment through the production of secreted molecules. This can, in turn, alter interaction strengths among species and modify community composition. However, the relative importance of such behaviours in determining the structure of complex communities is unknown. Here we show that single-locus changes affecting biofilm formation phenotypes in Bacillus subtilis modify community structure to the same extent as loss of an apex predator and even to a greater extent than loss of B. subtilis itself. These results, from experimentally manipulated multi- trophic microcosm assemblages, demonstrate that bacterial social traits are key modulators of the structure of their communities. Moreover, they show that intraspecific genetic varia- bility can be as important as strong trophic interactions in determining community dynamics. Microevolution may therefore be as important as species extinctions in shaping the response of microbial communities to environmental change.

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Single Cell Analysis of Contact Inhibition in Bacterial Inter-species Competition

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Microbial communities create an environment where competition for space and resources leads to rapid evolution of cooperation and competition mechanisms. Contact-dependent inhibition (CDI) and the Type VI secretion system (T6SS) are different mechanisms of contact-dependent toxin delivery. How the cost and efficacy of these systems influences growth of bacteria in microcolonies and biofilms is not well understood. This work quantifies single cell growth rates upon inhibitor and target cell interaction, in order to look at how the toxin systems affect microcolony spatial patterning. The nosocomial pathogen *Enterobacter cloacae* inhibits growth of the target *Escherichia coli*, delivering toxins with either its T6SS or CDI system. These target and inhibitor cell growth rates were used to parameterise an agent-based model using the Cell Modeller package, extended with a stochastic Gibson-Bruck algorithm. Our simulations suggest that inhibition creates spatial segregation of cell types as opposed to mixing of cell types without inhibition. This can occur with both strong and weak toxin activity and inhibition efficacy, suggesting that not only the killing of T6SS but also the weak CDI-dependent inhibition can create spatial segregation in mixed cultures. Our work supports the previously reported ideas that CDI is capable of being involved in kin discrimination, adhesion, signalling and biofilm formation, but open questions remain about the role of T6SS, with its predicted higher relative costs and strong killing effect. Understanding this interplay between cost and efficacy of toxin systems can help us to manipulate and control microbial systems.

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A novel *in vitro* interkingdom wound biofilm model can support the growth of anaerobic bacteria

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Background

Chronic wounds, such as diabetic foot ulcers, are commonly infected leading to recurrence and chronicity of the wounds, although this is not well characterised. We have previously developed a simple novel three-dimensional *in vitro* interkingdom wound biofilm model that has been used to characterise the response to wound washes. Here, the complexity of the model was increased to include anaerobes species found within our recent diabetic wound microbiome analysis.

Methods

These biofilm consortia consisted of the yeast, *Candida albicans*, the aerobes, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus hominis*, *Streptococcus agalactiae*, and *Corynebacterium simulans*. The anaerobes included were *Finegoldia magna*, *Anaerococcus vaginalis*, *Peptoniphilus gorbachii*, *Porphyromonas asaccharolytica*, and *Prevotella buccalis*. Biofilms were grown over a period of 9 days within a cellulose matrix hydrogel, with intermittent sampling. Compositional qPCR was used to enumerate each species within the biofilm.

Results

Overall, approximately 10^8 bacteria colonised the cellulose matrix, comprised of yeasts, aerobes and anaerobes. Aerobic bacteria were reduced by approximately 2 logs when the biofilm was grown in anaerobic conditions compared to 5% CO_2 or aerobically ($p<0.01$). Bacterial species were shown to coaggregate with the yeast and hyphae, supporting their adhesion and biofilm formation.

Conclusions

The addition of anaerobes to the wound model further reflects the environment *in vivo*, allowing this model to be used for accurate testing of antimicrobial agents. The ability of *C. albicans* to support interkingdom interactions, as well as anaerobes in a non-anoxic environment may explain why we observe elevated levels of anaerobes in these diabetic wounds.

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D- Amino acids do not trigger biofilm disassembly in *S. aureus* 8325-4 and *S. epidermidis* 1457.

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D-amino acids have been reported to interfere with biofilms and in this study we examined their effect on *S. epidermidis* and *S. aureus* biofilms. Specific *S. aureus* and *S. epidermidis* strains were selected and examined due to their biofilm forming capacity and their prevalence in healthcare settings. Biofilms formed by these strains were dispersed by sodium metaperiodate, indicating that they produce polysaccharide-type biofilms.

The crystal violet assay and spectrophotometry were used to quantify biofilm formation with an A_{492} of >0.17 defined as a biofilm positive phenotype. This threshold was used throughout the study to identify any metabolite that was capable of preventing or disassembling biofilm. Combinations of amino acids including tyrosine, tryptophan, methionine and phenylalanine were prepared and their effects examined. In addition, these amino acid mixtures and others were added to planktonic cells and over time examined microscopically to investigate any morphological changes.

D-amino acids did not prevent biofilm formation of *S. aureus* 8325-4 or *S. epidermidis* 1457 under any test condition or combination. Furthermore no combination or concentration was capable of dispersing mature biofilms of either species. Our investigations indicate that the effects of these compounds could be very strain specific and are in accordance with very recent reports which contradict early studies proposing the efficacy of these amino acids stereoisomers.

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***Candida albicans and Staphylococcus aureus* interactions within mycofilms enhance antimicrobial resistance.**

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Background: Candida albicans (Ca) and Staphylococcus aureus (Sa) are known to habitually co-exist and cause infection within various niche of the human host. This study aimed to characterize the interaction of these organisms within a complex biofilm community and determine their susceptibility to miconazole.

Methods: Ca SC5314 and Sa Newman strain were used throughout this study. *Ca* biofilm formation in the presence and absence of *Sa* was quantified using biofilm assays and live/dead qPCR. Representative biofilms were then visualized using scanning electron and confocal microscopy. Mono and dual-species biofilms sensitivity to miconazole was then assessed by MIC testing using both two and three-dimensional biofilm models. In addition, sensitivities to miconazole were characterised in vivo with the Galleria mellonella model.

Results: Data showed that *Ca* biofilms were able to support the growth and colonization of *Sa*. Biomass of the dual-species biofilm was significantly increased ($p<0.001$) throughout different phases of biofilm development. Compositionally, the quantity of *Sa* was significantly enhanced within the dual species biofilm compared to mono species ($p<0.01$). Microscopic analysis showed *Sa* micro-colonies adhered with *Ca* hyphae and ECM. Miconazole sensitivity was reduced in mono- and dual-species biofilms compared to planktonic counterparts. Dual-species biofilms were shown to be less susceptible to miconazole both *in vitro* and *in vivo*.

Conclusions: This data highlights the importance of how *Ca* can stimulate Sa biofilm formation as well as acting as a protective reservoir to hinder antimicrobial susceptibility. Further studies are merited to understand the complex relationship between these organisms.

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Copper Polyamide 11 antimicrobial composites for medical device applications.

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Direct incorporation of antimicrobial additive into the polymer matrix is a cost effective approach for the development of polymer/metal antimicrobial composites. Application of these antimicrobial composite systems for manufacturing the medical device addresses the issue of device related infections. In the present study, commercially available inorganic copper based additive, Plasticopper was incorporated into a Polyamide 11(PA 11) matrix during the polymer processing stage. These polymer composites were evaluated for their morphological, mechanical, antimicrobial and ion release properties. Isothermal crystallisation studies showed that the copper additive acted as a nucleating agent and promoted faster crystallisation. Short term mechanical studies confirmed that the incorporation of copper has reinforcing effect on the composites with 5 and 10% copper loadings and did not adversely affect the short-term mechanical performance of the polymer composites. These composite systems were shown to be active against *Escherichia coli* ATCC 8739 with >99.99% reduction in the bacterial population. Corresponding ion release profiles for these composites were encouraging and capable of long term antimicrobial activity.

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Analysis of 2-(2-hydroxylphenyl)-thiazole-4-carbaldehyde (IQS) Production by the Gene Products of the Five-gene Cluster *ambABCDE* and its Relationship with *P. aeruginosa* Quorum Sensing System.

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Pseudomonas aeruginosa is able to colonise several hosts by producing a variety of virulence factors and secondary metabolites. One of these is L-2-amino-4-methoxy-trans-3-butenoic acid (AMB). AMB biosynthesis is known to be directed by the gene products of the five-gene cluster *ambABCDE*. These genes have also been associated with the quorum sensing (QS) system of *P. aeruginosa* by producing the putative QS molecule, 2-(2-hydroxylphenyl)-thiazole-4-carbaldehyde (IQS). IQS has been described to activate the RhlR and PqsR systems in *ambB* mutants where pyocyanin production was restored under phosphate-limited conditions. To further investigate these findings, we created transcriptional fusions of *ambA* and *ambB* and tested the expression of these genes in a range of *amb* and QS mutants in both low and normal phosphate conditions. Expression of these genes was not altered when synthetic exogenous IQS was added to the cultures at concentrations up to 200 µM. We also created *phzA1* and *phzA2* transcriptional reporter fusions and analysed the expression and production of pyocyanin in these mutants under low phosphate conditions. Pyocyanin expression and production was unaltered compared to the wild type. The QS system has been directly correlated to motility and biofilm formation phenotypes. We tested all *ambABCDE* individual mutants for swimming, swarming, and twitching motility, as well as for the ability to form biofilms. Across all assays, there was also no difference among mutant and wild type strains. The relationship of the *ambABCDE* genes to IQS production and of the putative IQS signal molecule to QS is therefore unclear.

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A comparative metagenomic investigation of the root biofilm microbiome retrieved from Phragmites australis and Typha latifolia

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The role of plants and roots in the phytoremediation process has been clarified in detail. However, very little is known about the potential for bioremediation of microbial biofilms on root surfaces of aquatic plants. Here I describe a metagenomics investigation of the microbial biofilm associated with Phragmites australis and Typha latifolia roots, two aquatic plants commonly present in natural and artificial wetland. Root systems of plant species, rhizosphere soil and water were sampled from a natural wetland in southern Italy. Genomic DNA was extracted from all soil and water samples and from biofilm after its detachment from the root surfaces. From each DNA sample the 16S rDNA gene was amplified to construct an Illumina sequencing library. Biofilm microbiomes of Phragmites australis and Typha latifolia were compared each other and with rhizosphere and water microbiomes. The preliminary results showed that both biofilm and rhizosphere samples were largely represented by members of Proteobacteria and Bacteroidetes, while the phylum Acidobacteria was significantly depleted from biofilm preparations. Conversely, the phylum Cyanobacteria resulted increased. Closer inspection of the sequencing profiles revealed that Acidobacteria, Cloroflexi, Firmicutes, Verrucomicrobia, Actinobacteria and Nitrospirae were quantitatively more abundant in Phragmites australis biofilm than in Typha latifolia, whose biofilm microbiome was dominated by Bacteroidetes and Cyanobacteria. Together, our data suggest that, similar to the terrestrial plant microbiome, the biofilm microbiome of aquatic plants is not randomly assembled from the surrounding environment but rather, is the outcome of a selective process mediated, at least in part, by the host plant.

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Fibrinolytic protease from *Microbacterium* sp. reduces *Staphylococcus aureus* biofilm and inhibits its formation

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Biofilm is one of the survival strategies of bacteria aimed at avoiding the response of host organisms. Formation of this structured community is a complex process, regulated by interaction between both bacteria and host. It was shown that host plasma proteins, as well as extracellular proteases released by bacteria influence formation, remodelling and degradation of biofilm.

We have identified a fibrinolytic extracellular protease MfpA (*Microbacterial fibrinolytic protease A)*, produced by *Microbacterium* sp., which degrades fibrinogen and fibrin, the proteins that may serve as a scaffolding for the biofilm. The aim of the study was to investigate the influence of MfpA on biofilm made by *S. aureus*.

Biofilm was formed on high-binding polystyrene 96-well plates with surface pre-coated with fibrinogen, albumin, plasma solution, or buffer treated. Recombinant *S. aureus* LS-1 expressing GFP was cultured in tryptic soy broth and the formed biofilm was quantified by the measurement of fluorescence at 485/515 nm. The structure of biofilm was visualised with fluorescence microscopy.

We have shown that the MfpA reduces biofilm formation by *S. aureus* and disrupts earlier established biofilm. When bacteria were grown with the protease, the strongest inhibition of biofilm formation was observed for the surface pre-coated with fibrinogen. For plasma solution, albumin, and the buffer the effect was considerably smaller. For already established biofilms the reduction of the fibrinogen-based one was also the strongest. This indicates that the MfpA reduces biofilm by preferential digestion of fibrinogen, as a binding support, but also degrades staphylococcal proteins involved in biofilm formation.

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Development of an *in vivo* aerosol infection model employing encapsulated *Mycobacterium tuberculosis*.

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Tuberculosis, a contagious and often severe airborne disease, caused by the *Mycobacterium tuberculosis* (*Mtb*) bacterium, is a major cause of morbidity and mortality worldwide with an estimated 10.4 million incident cases occurring globally in 2015. The overwhelming majority of work undertaken to characterise *Mtb* in terms of *in vivo* infection and immunological studies relies on cultures grown in the presence of detergent to facilitate non-aggregated cultures. Growing the bacteria under these conditions results in a loss of the capsule layer which is believed to play an important role during natural transmission events.

At PHE, we have established the feasibility of preparing *Mtb* cultures such that capsule is retained during storage and following nebulisation for aerosol delivery. The retention of capsular material occurred through manipulation of growth conditions and screening of different nebulisation methods with the results verified by cryo-EM with immunogold labelling. *In vivo* work conducted at Containment Level 3 (CL3) demonstrates that aerosols containing a range of doses of capsule-positive *Mtb* can be delivered successfully to mice. In addition, we have shown that capsule-positive *Mtb* established a productive respiratory infection and that high within-group reproducibility was achieved for both the *Mtb* aerosol delivery (retained dose) and the subsequent early bacterial load (day 9 post-challenge) in the lungs.

By generating an infection model for *Mtb* disease that recapitulates natural infections to a greater degree, it may be possible to further understand the pathogenicity of the bacterium and evaluate different classes of antimicrobial interventions which have an impact on early-infection events.

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Investigating the mechanism of secretion of the moonlighting proteins enolase and fructose-1,6-bisphosphate aldolase (FBA) in *Neisseria meningitidis*

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Neisseria meningitidis is an obligate commensal of humans that colonizes the nasopharynx. It is also an important cause of serious diseases such as meningitis and sepsis. Several secretion systems have been shown to operate in meningococci, which enable the bacteria to modulate their cell surface and evade the human immune system. There is an increasing awareness of the contribution of moonlighting proteins to bacterial pathogenesis; these are secreted via unknown secretion mechanisms. The moonlighting proteins FBA and enolase were previously shown to localize to the surface of *N. meningitidis* (Knaust *et al.* 2007, Tunio *et al.* 2010, Shams *et al.* 2016). The mechanisms of secretion of both proteins were investigated in this study since the pathway of their translocation into meningococcal cell surface remains unclear. Specifically, the lysine residue 337 of meningococcal enolase was mutated by site-directed mutagenesis to investigate its role in enolase secretion since in *Escherichia coli* mutation of the corresponding residue has been reported to decrease export efficiency (Boel *et al.* 2004). The production of *N. meningitidis* MC58 *rpsL*$^{-}$ strains containing either wild type or mutated ectopic versions of the enolase was successful. Unmarked mutants lacking the native enolase gene using the two complemented strains were also successfully generated. Finally, whole-cell ELISA was optimised to investigate the contribution of several secretion-related or pathogenesis-related proteins to FBA and enolase surface localization. This analysis showed that the mutation of *pilQ, porA, skp, surA*, *hlyB*, and *cbbA*^{EctK354A} did not significantly affect the amount of FBA on the cell surface.

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Genomic comparative analysis of *Lactobacillus salivarius* EPS clusters and impact of carbohydrate availability on strains immuno-modulation activity

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Lactobacillus salivarius is a gut commensal with reputed probiotic properties, including the ability to modulate the host immune system. *Lactobacillus* cell wall components are implicated in immuno-modulation activity by interacting with Toll-Like Receptors (TLRs) on immune cells. Exopolysaccharide (EPS) is a carbohydrate structure present on the cell wall of some *Lactobacillus* species which is suggested to play a role in immuno-modulation activity. Environmental factors such as carbohydrate availability are expected to influence EPS structure. The resulting modification of the EPS structure and chemical composition could impact on *L. salivarius* immuno-modulation activity.

The aim of this study was to perform a comparative genomic analysis of *L. salivarius* EPS clusters to identify differences and similarities in EPS gene organisation in 34 strains. The impact of carbohydrate availability on EPS gene expression was measured by qPCR for 16 strains representing different EPS gene organisation types. The effect of carbohydrate availability on immuno-modulation activity was also examined by co-culturing *L. salivarius* strains, that were grown in 6 different carbohydrate-containing media, with THP-1 differentiated macrophages. Production of IL-6, IL-10 and TNF-α by macrophages were measured by ELISA.

Genomic comparative analysis of *L. salivarius* showed an exceptional variation in the gene composition of *L. salivarius* EPS clusters. This *L. salivarius* EPS genetic variability was shown to lead to the production of EPSs having different composition and conformation. These EPS structural variations may explain the observed differences in immune profiles of different strains.

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Adhesion of the Human Pathogen Staphylococcus Aureus to Mucin

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Human nasal Colonization with *Staphylococcus aureus* sets the stage for subsequent systemic infection. This study characterizes *S. aureus* adhesion to nasal mucus in vitro and observes the interaction of *S. aureus* with mucin by binding assay. *S. aureus* binding to cell-associated and cell-free mucus was greater than to non-mucin-coated well during log- phase. However, the pathogen showed significantly higher binding to mucin during stationary phase in comparison to log-phase cells. *S. aureus* mutant *srt*A adherence to mucin was not significantly different from wild-type, this means it may has not affect in binding. Pretreatment bacteria with trypsin reduced adherence because, that amplify the cause of binding to mucin by proteins component in *S. aureus*. Biotin –labelled mucin bound to surface proteins (~17kDa) of extracted *S. aureus*. However, Co-immunoprecipitation displayed different sizes with ~55KDa that was much bind with labelled mucin. These data suggest that a much adherence is present on the surface of *S. aureus* during stationary phase. Mass spectrometry analysis showed the second immunoglobulin-binding protein (Sbi) as a major surface protein co-precipitating with *S. aureus* proteins.

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Prevalence of Methicillin Resistant Staphylococcus aureus (MRSA) Infection In Inpatients In a Health Care Centre at Kathmandu, Nepal

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Background: Methicillin resistant Staphylococcus aureus (MRSA) infection is a major cause of infections worldwide. Nepal is a developing country with relatively less developed health care infrastructures and clinical practices. There are very few data on prevalence of MRSA infection in Nepal.

Methods: Three hundred and sixteen samples were collected from the patients who stayed for more than 48 hours in hospital and was further processed with standard microbiological methods. Sample exclusion criteria includes patients who had intranasal mupirocin or polysporin and oral antimicrobials for eradication of MRSA within past fourteen days. Mannitol Salt Agar was used as selective medium for Staphylococcus aureus. The antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI, 2007) guidelines.

Results: Growth was seen only in 79 out of 316 samples collected and S. aureus was found to be dominant organism for causing infections. Among 29 S. aureus isolates, 27.6% S. aureus was found to be methicillin resistant. The maximum number of S. aureus was isolated from the ICU ward. The number of MRSA and MSSA increased with the age of patient. The antibiogram of MRSA and MSSA showed great variance. The highest number of S. aureus was resistant to Amoxicillin (62.06%) and least resistant to Gentamycin (24.1%).

Conclusion: The prevalence of MRSA infection highlights the need of regular surveillance in order to control MRSA.

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Mutation of a 6 member *MNN1* gene family in *Candida albicans* using CRISPR-Cas9 to elucidate the role of fungal cell wall α-1,3-mannosides on the activation of the human immune response

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Candida albicans is the commonest serious human fungal pathogen causing between 250 000 and 400 000 deaths annually worldwide and around 100 million episodes of recurrent vaginitis [1]. The cell wall plays a critical role in its interaction with the host harbors many molecules that enable adherence to host cells and the activation of the host immune responses. Mannan is a major component of the outer surface of the cell wall and is critical for the adherence of the fungus to hosts cells and the activation of host immune responses. Mannans are synthesized by a series of families of glycosyl transferase enzymes.
Here we analyze the six-membered *MNN1* gene family that are predicted to act in the terminal stages of glycosylation by adding α-1,3-mannan residues to the side chains of N-linked mannans. Because these α-1,3-mannan residues represent the terminal sugars of the outer cell wall we predicted they may have major roles in host-fungus interactions of *C. albicans*. We created sextuple *MMN1-6* mutants by disrupting all twelve *MNN1* alleles using CRISPR-Cas9 genome editing technology [2]. Removal of the outermost N-linked α-1,3-mannosides resulted in alterations in yeast cell shape and in the profile of the immune response in macrophage cell cultures as well as human peripheral blood monocytes. These results suggest that the α-1,3-mannosides of *C. albicans* N-mannan is important in cell wall morphogenesis and immune recognition by cells of the innate immune system.

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2. Vyas., et al., 2015. Sci Adv

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Characterization of outer membrane vesicles from *Pasteurella multocida* strains recovered from different host species

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Pasteurella multocida is a Gram-negative bacterium that resides in the upper respiratory tract of domesticated animals. It is the aetiological agent of fowl cholera in poultry, enzootic pneumonia in cattle, sheep and pigs, atrophic rhinitis in pigs, and haemorrhagic septicaemia in cattle and buffaloes. The ability of *P. multocida* to cause disease in different host species is attributed to the presence of virulence factors such as capsule, lipopolysaccharides (LPS), pili, outer membrane proteins (OMPs), enzymes and toxins. Outer membrane vesicles (OMVs) are derived from the outer membranes (OM) of Gram-negative bacteria and contain OM components together with periplasmic proteins. Hence, OMVs have the capacity to transport active and specific proteins into the extracellular milieu. Using various techniques this study aimed to compare and characterize the OMVs produced by eight *P. multocida* isolates recovered from diseased cattle, sheep, pigs and poultry. OMVs isolated by serial centrifugation were observed by transmission electron microscopy to have an average diameter of 24±10 nm. SDS-PAGE analysis indicated that OMVs were enriched with OMPs and LPS. Proteomic analysis carried out by in-solution digestion coupled with MS/MS identified key virulence proteins such as OmpA, HgbA, TadD and phospholipase A within OMVs. A total of 170 OMV proteins were identified in the eight *P. multocida* isolates and these proteins have roles in OM biogenesis, iron-uptake, adherence and enzymatic activity. Our findings highlight the potentially important role that OMVs play in disease pathogenesis and also identifies proteins for further evaluation as putative virulence factors and candidate vaccines antigens.

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Eating the poison: characterisation of the molecular basis of antimicrobial peptide resistance in pathogenic Escherichia coli.

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Human pathogenic bacteria have evolved a number of mechanisms to resist cationic antimicrobial peptides (CAMPs), a branch of the innate human immune response. CAMPs gain access to the membrane of bacteria to exert their bacteriocidal effect, however, bacteria have evolved ATP-binding cassette (ABC) transporters called Sap and Yej that can recognise CAMPs and actively sequester them into the cell for degradation, thus bypassing their bacteriocidal effect at the membrane. Using an interdisciplinary approach, spanning structural biology, molecular modelling, molecular biology and microbiology we aim to understand how the Sap and Yej transporters have evolved to recognise CAMPs, which are unusually large and complex substrates for this family of ABC transporters and how CAMP degradation occurs after CAMP binding and transport. Interestingly, use of the Yej transporter comes at a cost, as it appears to be the sole route by which the peptide-based antibiotic Microcin C enters the cell, which leads to cell death through inhibition of aspartyl-tRNA synthetase. We will present progress on the expression and purification of SapA and YejA, the substrate binding proteins (SBPs) of the two ABC transporters and attempts to crystallise these proteins to elucidate how CAMP and Microcin C recognition is achieved.

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Identification, genetic and function characterisation of a novel Lawsonia intracellularis surface protein

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

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PlpA plays a role in membrane integrity in *Escherichia coli*

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The Gram-negative outer membrane is an asymmetric lipid bilayer composed of lipopolysaccharide and phospholipid decorated with integral outer membrane proteins and peripheral lipoproteins. The lipopolysaccharide and protein components are synthesised in the cytoplasm and trafficked to the outer membrane by known, dedicated systems. However, phospholipid trafficking and maintenance of the outer membrane remains unclear. PlpA is a conserved outer membrane lipoprotein with an unresolved structure and function. Here we present its solution structure, both free and inserted into outer-membrane mimicking micelles. This reveals PlpA is composed of two BON domains that form an interconnected opposing pair. This is an unprecedented finding with functional repercussions. The C-terminal BON domain interfaces phosphatidylglycerol-based membrane surfaces. Loss of PlpA resulted in impaired outer membrane barrier function indicated by sensitivity to SDS, vancomycin and EDTA. Further to this, fluorescently labelled PlpA was found to be distributed throughout the outer membrane, but localised to the septum during division. While the precise function of PlpA remains unclear, these data suggest that it is clearly involved in maintenance of the *Escherichia coli* outer membrane.

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Molecular dissection of an SRP dependent signal sequence

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In Escherichia coli periplasmic and outer membrane proteins are translocated across the cytoplasmic membrane by the Sec machinery whose central component, SecYEG, forms a protein conducting channel in the membrane. Proteins can be translocated through SecYEG by two different mechanisms: (i) a strictly cotranslational mechanism, or (ii) a posttranslational mechanism.

Proteins are recognized by the Sec machinery by virtue of a cleavable N terminal signal sequence, which contains a hydrophobic core flanked by shorter N and C terminal domains. Signal sequences that are recognised by the signal recognition particle (SRP) are targeted for cotranslational translocation. Hydrophobicity is thought to be the determining factor within the signal sequence for SRP recognition early in translation. However, many very hydrophobic signal sequences are not recognised by the SRP, suggesting that features other than hydrophobicity may affect SRP dependence.

We made a series of synthetic signal sequences by replacing the N- and C-terminal domains of an SRP dependent signal sequence with those of SecA dependent signal sequences with highly hydrophobic cores. In order to determine whether these signal sequences were capable of targeting proteins for SRP-dependent translocation we fused these hybrid signal sequences to thioredoxin, a normally cytoplasmic protein that can only be efficiently exported across the cytoplasmic membrane by the SRP-dependent pathway. We then systematically mutagenized the hybrid signal sequence in order to determine which residues were important for SRP recognition. We also set up a genetic screen to select for signal sequences that are targeted by the SRP.

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Outer membrane lipid homeostasis via retrograde phospholipid transport

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The outer membrane (OM) of Gram-negative bacteria comprises lipopolysaccharides (LPS) and phospholipids (PLs) in the outer and inner leaflets, respectively. This asymmetric distribution of lipids renders the OM a very effective permeability barrier against toxic compounds, including bile salts and antibiotics. To build a stable OM with the requisite lipid asymmetry, the transport and assembly of LPS, PLs and OM proteins in the OM must be well coordinated. How homeostasis at the OM is achieved, however, is currently unknown. The Tol-Pal complex is a trans-envelope machine that is important for OM stability. Despite extensive studies on this complex over the last 30 years, its physiological function remains elusive. Here, we establish the function of the Tol-Pal complex in OM lipid homeostasis. We show that cells lacking the complex contain normal LPS levels but accumulate excess PLs in the OM. This imbalance in OM lipids (higher PL:LPS ratios) can account for all known phenotypes observed in *tol-pal* mutant strains. We further demonstrate that retrograde PL transport is defective in cells lacking a functional Tol-Pal complex. Our work suggests a model where cells ensure assembly of a stable OM by maintaining an over flux of PLs to the OM (presumably to occupy space not taken up by other components) only to return the excess PLs to the inner membrane via Tol-Pal-mediated retrograde transport.

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Flipping trehalose monomycolate across the inner membrane of mycobacteria

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The mycobacterial outer membrane (OM) is characterized by the presence of mycolic acids, which are C_{60}-C_{90} long, branched chain fatty acids, either existing in the forms of trehalose mono- and diesters, or covalently attached to peptidoglycan via arabinogalactan polysaccharides. Being the major component in the OM, mycolic acids make this bilayer extremely hydrophobic and render the membrane impervious to many antibiotics. Mycolic acids are synthesized in the cell as trehalose monomycolates (TMMs), and have to be translocated across the inner membrane (IM) and the aqueous periplasm before being functionalized onto the cell wall. While the biosynthetic pathway of TMM and the final steps of assembly at the OM have been well characterized, how TMM is transported across the IM and periplasm are still not clear. Recently, an essential IM protein MmpL3 has been implicated in TMM transport across the cell envelope and is believed to be inhibited by multiple pharmacophores. Here, we present direct biochemical evidence for the function of MmpL3 in flipping TMM across the IM. Furthermore, we demonstrate that a couple of potential MmpL3 inhibitors tested directly target the TMM flippase. Our work provides fundamental insights into mycolic acid transport and validates MmpL3 as a viable target for the development of new antibiotics against mycobacterial infections.

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Role of IcaC in *Staphylococcus aureus* biofilm formation

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Staphylococcus aureus and *Staphylococcus epidermidis* cause serious infections through their ability to form biofilms on in-dwelling medical devices. A major component of staphylococcal biofilms is ß-1,6-linked poly-N-actylglucosamine (PNAG), produced and exported by the IcaADBC proteins. In *S. aureus* and *S. epidermidis,* PNAG is partially N-deacetylated and O-succinylated and these modifications are likely to be important for biofilm function, but the precise molecular mechanisms by which they occur are unknown. Here, we use bioinformatics and molecular techniques to investigate the role of IcaC as a putative succinyltransferase responsible for the O-succinylation of PNAG.

IcaC is an intrinsic membrane protein with a predicted 10 transmembrane helices. Although originally thought to transport PNAG, recent bioinformatics analysis of PNAG-exporting clusters in a range of bacteria suggests that IcaC does not play a direct role in transport. Further, analysis of staphylococcal sequences places IcaC in a large family of acytransferases, suggesting that its true function may be O-succinylation of PNAG, for which an enzyme has not previously been identified. Functional analysis of purified IcaC protein will test this hypothesis.

Disruption of *icaC* has been shown to abolish biofilm formation both in laboratory strains and in clinical isolates of *S. aureus*. Functional characterisation of IcaC could therefore lead to identification of a novel Staphylococcus-specific therapeutic target, as well as a greater understanding of staphylococcal biofilm formation and its role in persistent infection.

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Bacillus subtilis cell division and PBPs

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Bacterial cell division involves the dynamic assembly of a diverse set of proteins that coordinate the invagination of the cell membrane and synthesis of cell wall material to divide the daughter cells. A key cell division protein in *Bacillus subtilis* is PBP 2B, a pencillin binding protein mediates the assembly of other proteins and is thought to provide a unique biochemical activity. We find that a catalytically inactive mutant of PBP 2B supports cell division, but in this background the normally dispensable PBP 3 becomes essential. Further phenotypic analysis of *pbpC* mutants (the gene encoding PBP 3) confirms that PBP 2B has both a structural role in the assembly of the division complex as well as a redundant biochemical activity acting is septum formation. Surprisingly this activity can be provided by PBP 3 apparently acting from outside the complex as the incative PBP 2B has to be present.

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The role of apolipoprotein N-acyl transferase, Lnt, in the lipidation of Factor H binding protein of Neisseria meningitidis strain MC58 and its potential as a drug target.

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Background and Purpose
The level of cell surface expression of the meningococcal vaccine antigen, Factor H binding protein (FHbp) varies between and within strains and this limits the breadth of strains that can be targeted by FHbp-based vaccines. The molecular pathway dictating expression of FHbp at the cell surface, including its lipidation, sorting to the outer membrane and export, and the potential regulation of this pathway have not been investigated until now. This knowledge will aid our evaluation of FHbp vaccines.
Experimental Approach
A meningococcal transposon library was screened by whole cell immuno-dot blotting using an anti-FHbp antibody to identify a mutant with reduced binding and the disrupted gene was determined.
Key Results
In a mutant with markedly reduced binding, the transposon was located in the lnt gene which encodes apolipoprotein N-acyl transferase, Lnt, responsible for the addition of the third fatty acid to apolipoproteins prior to their sorting to the outer membrane. We provide data indicating that in the Lnt mutant, FHbp is diacylated and its expression within the cell is reduced 10 fold, partly due to inhibition of transcription. Furthermore the Lnt mutant showed 64 fold and 16 fold increase in susceptibility to Rifampicin and Ciprofloxacin respectively.
Conclusion and Implications
We speculate that the inefficient sorting of diacylated FHbp in the meningococcus results in its accumulation in the periplasm inducing an envelope stress response to down-regulate its expression. We propose Lnt as a potential novel drug target for combination therapy with antibiotics.

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A ROLE FOR PROTEOBACTERIAL MAMMALIAN CELL ENTRY DOMAINS IN PHOSPHOLIPID TRAFFICKING

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Mammalian cell entry (MCE) domains are so called due to the reported ability of an *Escherichia coli* strain harbouring the *mce1* gene from *Mycobacterium tuberculosis* to invade mammalian cells. Bioinformatic analyses presented here demonstrated that proteins containing a single MCE domain are widespread in bacteria and that proteins containing multiple MCE domains are specific to and have evolved within Proteobacteria. Gene neighbourhood analyses revealed that MCE domain containing proteins are components of transporters and that multi MCE domain containing proteins constitute a novel type of transporter. *E. coli* was shown to harbour three MCE domain containing proteins: the single MCE domain protein MlaD and two multi-domain proteins PqiB and YebT. All three proteins were shown to locate to the inner membrane. Phenotypes on detergents and vancomycin revealed that their functions are overlapping yet distinct. All three proteins bind the major phospholipids phosphatidylethanolamine and phosphatidyglycerol. These findings suggest that MCE domain containing proteins in Proteobacteria are involved in phospholipid trafficking and a related to maintenance of the cell envelope.

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IscR regulation of type 3 fimbriae expression in *Klebsiella pneumoniae* CG43

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In *K. pneumoniae*, we have previously shown that IscR, an Fe-S cluster-containing transcriptional factor, plays a dual role in control of capsular polysaccharide biosynthesis and iron-acquisition systems via switches between its holo and apo forms. In this study, the effect of IscR on biofilm formation and type 3 fimbriae expression was investigated. A higher biofilm formation was found in D*iscR* or $iscR_{3CA}$, a strain expressing mutated IscR mimicked apo-IscR, suggesting that holo-IscR represses the biofilm formation. Based on type 3 fimbriae is the major factor to affect the biofilm formation, we also found that production of the major subunit of type 3 fimbriae, MrkA, was increased in D*iscR* or $iscR_{3CA}$ strain at either translational or transcriptional level. However, repression of IscR in the type 3 fimbriae expression is indirect. To further understand the regulatory mechanism of IscR, the effect of IscR on *mrkHIJ* expression, that encode cyclic di-GMP (c-di-GMP)-related regulatory proteins to control the type 3 fimbriae expression, was studied. We found that holo-IscR could directly repress the *mrkHI* transcription, indicating that MrkHI is required for IscR regulation in the type 3 fimbriae expression. Finally, deletion of *iscR* could attenuate *K. pneumoniae* virulence in the peritonitis model of mouse infection, while the absence of the [2Fe-2S] cluster of IscR has no affect on *K. pneumoniae* virulence during the infection. Take together, we demonstrated that the underlying mechanism of the [2Fe-2S] cluster of IscR in control of type 3 fimbriae expression and the effect on *K. pneumoniae* pathogenesis.

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O-antigen modifying enzymes in the bacterial pathogen *Salmonella*

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Salmonella enterica serovar Typhimurium is the leading cause of gastroenteritis worldwide. Its outer membrane contains an abundance of lipopolysaccharide (LPS), of which the O-antigen is the outermost component. As the O-antigen is exposed to the host immune system, altering its structure by the addition of acetyl groups can contribute to the ability of *Salmonella* to evade host immunity. $GtrC^{BTP1}$ and OafA are inner membrane bound O-antigen acetyltransferases that function on the periplasmic side of the inner membrane to acetylate the O-antigen during its biosynthesis. It is as yet unclear how these proteins orchestrate acyl group transport into the periplasm and transfer onto the O-antigen. This research therefore hopes to elucidate the mechanism of O-antigen acetylation through structure/function analysis of $GtrC^{BTP1}$ and OafA. We have identified three domains within these proteins as well as conserved residues likely to be important for protein function. Initial focus has been on the C-terminal periplasmic SNGH hydrolase domain, predicted to be responsible for direct transfer of acetyl groups onto specific O-antigen monosaccharides. OafA SGNH hydrolase domain expression and purification has been achieved, *in vitro* catalytic activity of the domain has been shown and NMR analysis of C-terminal OafA has indicated that the expressed protein is structured. These findings provide a platform for further analysis to discover the mechanism of action of these little studied proteins. Understanding the biological process of O-antigen acetylation could allow manipulation of this and other similar systems, creating a repertoire of possibilities for disease control or carbohydrate engineering.

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Multiple sensory input into the biofilm matrix-controlling diguanylate cyclase DgcE of *Escherichia coli*

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Bacteria often form multicellular aggregates accompanied by the production of extracellular matrix components, which provide enhanced stress resistance. In most bacteria, biofilm formation is promoted by the second messenger c-di-GMP, which in *E. coli* K-12 is produced by 12 diguanylate cyclases (DGCs, with GGDEF domains) and degraded by 13 phosphodiesterases (PDEs, with EAL domain) (1). For synthesis of biofilm matrix components (amyloid curli fibres and the exopolysaccharide cellulose) the DGC DgcE serves as the essenial top-level trigger. DgcE shows basal expression in growing cells and additional Sigma-S-dependent induction during entry into stationary phase (2) and consists of several domains: a N-terminal transmembrane segment (including a MASE1 domain) followed by three PAS/PAC domains, a GGDEF domain and a degenerate EAL domain. Its complex structure as well as its crucial role in biofilm promotion suggested multiple signal-integration by DgcE necessary to ensure accurate temporal and spatial coordination of biofilm matrix production. Chromosomal deletion of single domains revealed activating and inhibitory impacts of specific domains. Furthermore, we found two additional factors required for DgcE activation. These interact with each other and one directly interacts with DgcE. Westernblot analysis of C-terminally 3xFLAG-tagged DgcE variants showed rapid and excessive proteolytic processing, whereas a DgcE version lacking the membrane-inserted N-terminus was more stable. In conclusion, we show that several processes are integrated in the regulation of DgcE activity including activation by other proteins and proteolytic events.

(1) Povolotsky, T.L., and R. Hengge (2015) J. Bacteriol. 198: 111-126.

(2) Sommerfeldt et al. (2009) Microbiology 155, 1318-1331

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Cell Wall Biogenesis in *Bordetella pertussis*: Bvg-dependent Essentiality of Cell Wall Synthesis Genes Revealed by TraDIS.

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The identification of essential genes for bacterial growth reveals much about their basic physiology. *Bordetella pertussis*, the causative agent of whooping cough, adopts both virulent and avirulent states through the activity of the two component system, Bvg.

The essential genes for *Bordetella pertussis* growth in vitro were defined. As expected, many of the genes identified as essential for growth in other bacteria were also essential for *B. pertussis.* However, the essentiality of some genes was Bvg-dependent. Key cell wall biosynthesis genes were essential for the avirulent, Bvg minus phase but not the virulent, Bvg plus phase. In addition, mutations in other cell wall biosynthesis genes produced greater fitness costs for the Bvg minus phase compared to the Bvg plus phase. Bvg minus phase growth was more susceptible than Bvg plus phase growth to the cell wall disrupting antibiotic ampicillin demonstrating the increased susceptibility of the Bvg minus phase to disruption of cell wall synthesis.

The conditional essentiality of cell wall biosynthesis genes demonstrates that this fundamental process differs between the Bvg phases in *B. pertussis* and is more susceptible to disruption in the Bvg minus phase. Bvg plus phase growth is relatively slow compared to that of the Bvg minus phase. Slow growth of virulent phase bacteria compared to their avirulent state is often attributed to the metabolic cost of expressing virulence factors. However, for *B. pertussis* we suggest that slow growth may be a protective response to protect against cell wall disrupting factors such as host antimicrobial peptides.

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Comparative transcriptomic studies of *Bifidobacterium longum* subsp. *infantis* iron-responsive genes

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Iron is a vital element for almost all organisms as it is utilised in fundamental cellular processes. Within mammals the majority of iron is located within insoluble complexes, composed of heme proteins and organic ligands (e.g. haemoglobin, transferrin and lactoferrin), which serves to restrict iron bioavailability. To overcome these iron-limiting conditions, bacteria have developed sophisticated strategies to sequester extracellular iron and maintain iron homeostasis. Whist iron uptake systems in pathogens has been well studied, our understanding of these processes within members of the gut microbiota are limited. *Bifidobacterium* are Gram-positive commensal bacteria, which play an important role in gut homeostasis, immune system development and colonisation resistance against pathogens. In this current study we sought to investigate the iron uptake systems in *B. longum* (subsp. *infantis*) through a comparative transcriptomic approach. Anaerobic cultures were grown under iron-deficient or iron-replete conditions (free-iron and iron-loaded lactoferrin), achieved via augmentation with the iron chelator 2,2'-bypyridyl +/- $FeCl_3$. RNAseq (Illumina HiSeq) and analysis was performed on cells harvested at different growth phases (lag, mid-log, and stationary) and the gene transcription profiles compared. These data will allow us to identify iron-responsive genes and decipher the pathways which mediate *Bifidobacterium* iron uptake, thus increasing our understanding of strategies required for optimal colonisation within the host. Subsequent *in vitro* and *in vivo* studies will focus on understanding these pathways in the context of critical colonisation resistance to enteric pathogens; *E. coli* and *Salmonella,* potentially identifying novel bifidobacterial beneficial traits, and thus therapeutic approaches, in this microbiota genus.

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Dissecting cell division in the human pathogen *Staphylococcus aureus.*

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Bacterial cell division is a fundamental process mediated by a large collection of proteins, collectively called the divisome. The divisome is responsible for the appropriate synthesis of new cell wall peptidoglycan to produce the septum, allowing the production of two new daughter cells. Although divisome components have been identified, their precise roles are not well understood.

In *Staphylococcus aureus*, cell division components, including EzrA, have been identified by a bacterial two-hybrid analysis. EzrA, a membrane-associated protein, interacts with both cytoplasmic proteins and those with periplasmic domains. It is therefore proposed to act as an interface between FtsZ in the cytoplasm and PBPs in the membrane forming a scaffold for other cell division components.

In this study, a combination of protein labelling and super-resolution microscopy approaches have been used to study the architecture of the cell division process in *S. aureus*. Conventional wide-field fluorescence microscopy shows EzrA and FtsZ as uniform rings at the division site. Super-resolution fluorescence microscopy of a strain, in which the only copy of EzrA was tagged with a fluorophore, revealed that EzrA does not form a homogenous ring but it is rather a collection of 'patches' distributed at the division site. In order to look at the architecture of the cell division process as a whole we are studying molecular level resolution of FtsZ (cytoplasmic component), through EzrA (membrane component) to PBPs and the product of the division machinery, that is peptidoglycan.

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Identifying Transcriptional Regulators of Mannan Biosynthesis in *Candida albicans*

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The outer mannan-rich layer in the cell wall of pathogenic fungus *Candida albicans* is complex, heterogeneous and differs in chemistry in different cell morphotypes. Many of the mannosyltransferases that collectively assemble the *O*- and *N*-linked cell wall mannans are encoded by multigene families; however, the transcriptional regulatory circuits for these enzymes have not been elucidated. Understanding this transcriptional regulation is important because the outer mannan layer is vital for many essential cellular functions and for host-pathogen interactions.

O-mannan is a linear structure composed of α-mannoses, whilst *N*-mannan is composed of a highly conserved core structure, to which is added fungal-specific extended and highly branched structure of mannan in different linkages. Considering these pathways involve concerted actions of many enzymes, often acting as a complex or multimers, we might expect multiple levels of regulations for this process. In this study we aim to identify the transcriptional regulators of mannosylation genes in *C. albicans.* We screened published metadata expression sets to identify putative regulators and orthologs in *C. albicans* for known regulators in *S. cerevisiae* were mapped. PathoYeastract was used to identify the regulators, based on promoter binding site predictions. We also screened mutant libraries for glycosylation defects. Candidate transcription factors are being further verified for their role in mannan biosynthesis regulation using genetic and biochemical approaches. Together we aim to elucidate the transcriptional regulatory network for fungal glycosylation and how this network is influenced by and modifies the cell wall in response to physiological and antibiotic stress, and immune system challenges.

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Altered urinary microbiota in Prostatitis

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The microbiome of the male urogenital tract is poorly described, but it has been suggested that the composition of bacterial communities in the urinary tract might affect the risk of prostatitis. To analyze the urogenital microbiota associated with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and clinical healthy control group, a qualitative and quantitative analysis of the bacterial diversity in the urethra and in expressed prostatic secretions (EPS) of 10 CP/CPPS and 10 healthy individuals was performed, using a 454 high-throughput pyrosequencing analysis. Based on a total of 100,931 bacterial 16S rRNA gene sequence reads, diversity indicators and statistical analysis using two-sided Welch's t-test for the comparison of two independent groups (health control and CP/CPPS patient). Proteobacteria were more dominant in CP/CPPS patients, whereas Firmicutes dominated in clinical healthy control. These observations indicate that the prostatitis is associated with alterations in the composition of the urogenital microbiota between bacterial colonization, progression, exacerbation, and clinical outcomes.

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Morphological and Genetic Characterization of Economically Important Aspergilli

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Aspergillus is the best-known and most-studied fungal genus due to the importance of several of its species being mycotoxins producers, animal and plant pathogens as well as their wide use in industry and research. For present study, eighteen strains of Aspergillus were characterized using polyphasic taxonomic approach. Initially, species were identified on the basis of morphological characters and then identification was confirmed using sequence analysis of the Internal Transcribed Spacer (ITS) region of rDNA and partial calmodulin gene (CAL). Phylogenetic analysis of strains was also carried out using the nucleotide sequence of both ITS and CAL gene. Based on morphology as well as nucleotide sequences, strains of Aspergillus were categorized into five different taxonomic groups. Five species of *A. niger* group that are characterized for present study are *A. niger*, *A. awamorii*, *A. tubingensis, A. welwitschiae, A. neoniger* and *A. phoenicis*. Two isolates each of *A. flavus* from *Aspergillus flavus* group and *A. fumigatus* from *Aspergillus fumigatus* group; one isolate each of *A. terreus* from *Aspergillus terreus* group and *A. tamarii* from *Aspergillus tamarii* group were characterized. BLAST results using ITS and CAL nucleotide sequences revealed 99-100% identity with the many of their respective strains deposited to GenBank. Phylogenetic analysis of ITS based results revealed lack of clear distinction amongst morphologically similar isolates however nucleotide sequence of CAL gene grouped morphologically similar strains in same clade. Present study concludes that proper identification using polyphasic approach is indeed requirement that will contribute information in stable taxonomy and nomenclature of Aspergillus group.

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Microevolution during persistent *Campylobacter jejuni* infection in an immunocompromised host

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Campylobacter, the most common cause of food poisoning in the UK, typically presents as an acute bout of gastroenteritis that resolves within a week and rarely necessitates treatment. However, in immunocompromised individuals the infection can be more severe or prolonged. A patient with hypogammaglobulinaemia suffered from persistent campylobacteriosis for 15 years. Laboratory typing performed over the course of infection previously revealed a range of serotypes. Despite the variable laboratory evidence, it was hypothesised that this prolonged infection was caused by a single strain of *C. jejuni* which the patient was unable to clear, rather than multiple instances of re-infection. Twenty-five isolates spanning the time period of the infection (2001-2016) were recovered and whole genome sequenced, alongside undergoing comprehensive antibiotic susceptibility testing. The genomes revealed that the isolates belonged to a single, clonal population which had undergone microevolution over time, with root-to-tip analysis showing a strong temporal signal. The majority of observed antibiotic resistance could be predicted from the genome. Changes in phenotypic resistance to erythromycin over the course of infection could be tracked by sequential mutations in the multi-copy 23S rRNA gene, with the level of resistance corresponding to the number of mutated copies of the gene. Whole genome sequencing (WGS) is a reliable tool for distinguishing closely related isolates of bacteria, even isolates from a single infection. WGS can also be utilised to predict antibiotic resistance and help elucidate the mechanism underlying changes to antimicrobial susceptibility over time.

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In situ metagenomic sequencing of bromeliad plant phytotelmata using the Oxford Nanopore MinION.

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The rare and endangered bromeliad plant, Glomeropitcainia erectiflora is found in unique elfin forest habitat on Trinidad two highest peaks, above 600 metres. The bracts between the leaves of the plant (phytotelma) are home to a host of organisms including the critically endangered and endemic golden tree frog. This highly humid and unusual environment offers an interesting and unexplored habitat for microorganisms. Water samples were collected from the phytotelmata of a number of these bromeliads and the DNA isolated. An Oxford Nanopore MinION was taken to the William Beebe Tropical Research Centre, in Trinidad, and used to sequence the DNA samples in the field. Samples were also returned to the University of Strathclyde and sequencing repeated in a laboratory. The results were then combined for analysis. Overall, more than 75,000 sequences were gathered, making up 39MBs of data. Around 27,000 reads passed quality controls. These reads where then processed through a range of pipelines including MG-RAST and MEGAN. From this the species diversity, phylogeny and feature predictions could be made. As expected, there are a large number of unidentified sequences, indicating that the phytotelmata of G. erectiflora are a diverse and dynamic habitant. This was a challenging experiment to perform in the field, with many setbacks but provided an interesting development in where DNA sequencing can be carried out and intriguing set of results to be analysed.

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Establishing method selection criteria for meta-genomic sequence analysis using high-throughput sequence simulators

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Background
The revolution in next-generation sequencing (NGS) technologies has enabled a step-change in the way that sequence data is collected and used in Biology including in meta-genomics, the sequencing of mixed source nucleic acid samples. The increasing quantity of meta-genomic sequence data being generated and the diversity of its application area requires highly optimised and computationally scalable solutions to process and interpret these data.

Methods
We present a comparative evaluation of meta-genomic analysis methods in which we use sequence simulators to generate gold-standard data against which to benchmark the efficacy of the methods. We use our method to develop an approach to estimate errors in taxonomic sequence assignment by perturbing the underlying taxonomic trees used in our simulations.

Results
Our method demonstrates the high dependency of taxonomic classification success and accuracy on the information present in the reference database. We also present an evaluation of the effects of selecting variable marker gene regions on the discriminatory power of OTU clustering. Using results from these quantitative analyses and considering the usability, functionality and compatibility of the methods we present a novel pipeline for meta-genomic analysis for both targeted and untargeted studies.

Conclusion
We present a novel approach to benchmark downstream meta-genomics analysis methods using sequence mixture simulation and a novel analysis pipeline with improved accuracy for taxonomic classification.

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CRISPR/Cas defences against bacteriophage infection are not detectable in El Tor lineages of *Vibrio cholerae*

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The dissemination of virulence within and amongst populations of *Vibrio cholerae* is dependent upon horizontal gene transfer (HGT) of genetic determinants, including the bacteriophage CTXϕ. However, bacteria have developed defensive mechanisms against HGT, such as CRISPR/Cas systems, to protect against infection by bacteriophages, plasmids, and the genomic insertion of foreign genetic elements. We have studied the evolution and distribution of CRISPR/Cas systems across a large dataset of *V. cholerae* genome sequences. These genomes encompass both pathogenic and non-pathogenic *V. cholerae* and include multiple strains genotyped as the O1 El Tor biotype, responsible for the current cholera pandemic. Although we identify CRISPR/Cas systems in environmental and non-El Tor lineages of *V. cholerae*, these systems are not detected in the strains genotyped as El Tor in our dataset. Since these pandemic strains are likely to have evolved genetically to survive in the context of a human host, our data suggest that the El Tor lineage is either under weaker selective pressure from bacteriophages to retain CRISPR/Cas defensive elements, or that the loss of CRISPR/Cas may be permissive to the horizontal exchange of genetic material and pathogenicity factors. We have studied the structure of CRISPR/Cas systems across our phylogeny, and observe that the spacer composition of CRISPR arrays varies, even between closely-related strains. These data mirror the phylogenetic relationship between strains, and we believe that these data reflect the order in which closely-related *V. cholerae* were exposed to bacteriophages.

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SnapperDB: Scalable integration of whole genome sequencing data of bacterial isolates for routine population analysis in public health microbiology.

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Public Health England has embarked on the implementation of high-throughput, real-time sequencing for the surveillance of several human pathogens and aims to leverage the high discriminatory power of single nucleotide polymorphisms (SNPs) to detect linked cases and outbreaks. Analysis of bacterial populations often requires re-computing of variants across all isolates, which is not feasible in rapidly growing, large datasets. As routine whole genome sequencing of isolates becomes a reality scalable data storage is required.

Here we present SnapperDB, a set of tools to store bacterial variant data to facilitate reproducible and scalable analysis of bacterial populations. SnapperDB takes as its input (i) a set of genome sequences and a reference genome or (ii) a VCF (Variant Call Format) file. In the former case, SnapperDB generates a VCF file by calling third-party mapping (BWA or Bowtie) and variant-calling (GATK or MPileup) software, integrated in the software package PHEnix. Variant distribution, as well as annotation for each variant, is stored in a PostgreSQL database. SnapperDB can be queried to output high quality variant positions for a selection of strains in FASTA format for phylogenetic inference.

SnapperDB also maintains a SNP distance matrix for all pairwise combinations of strains within the database. Using hierarchical single linkage clustering we can derive an isolate-level nomenclature for each genome sequence allowing efficient searching of the population and automated cluster detection. This "SNP address" has become the primary whole genome sequencing result for the surveillance of food-borne pathogens in England.

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Genome analysis of malonate, sialic acid and Indole metabolism in *Cronobacter* spp. strains.

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The genus *Cronobacter* are opportunistic pathogens. This genus is associated with neonatal and immunocompromised adults infections causing meningitis, necrotizing enterocolitis (NEC) and bacteraemia in neonates. *Cronobacter* strains were studied phenotypically to investigate their ability to produce indole, malonate metabolism and utilize sialic acid, and to linked these with their molecular characteristics. A total of 54 *Cronobacter* spp., including food and environmental isolates were used in this study. *C. muytjensii, C. dublinensis* strains and the single *C. condimenti* strain were able to produce indole. However, *C. sakazakii, C. malonaticus, C. turicensis, C. universalis* and *C. universalis* strains were negative for this test. *C. malonaticus*, *C. muytjensii C. universalis, C. dublinensis* strains and single *C. condiment* strain were unable to utilize sialic acid. However, *C. sakazakii* strains and some of *C. turicensis* strains were capable to utilize sialic acid. *C. malonaticus, C. universalis, C. turicensis* and *C. muytjensii* strains were able to utilize malonate. All *C. sakazakii* strains were negative for malonate utilization, but two strains 1845 and 1881 were able to utilize malonate. *C. dublinensis* strains were divided into two separate groups on the basis of malonate utilization. All strains were positive for the genes which are responsible for these traits, except for negative strains that lacked these genes. Therefore, for this dataset it is possible to differentiate and distinguish the seven species of *Cronobacter* as well as to characterize and distinguish of *C. dublinensis* strains to the subspecies level using genomic clusters and sialic acid, malonate and indole tests.

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Phylogenetic and comparative genomic analyses of *Fusobacterium necrophorum*: The cause of Lemierre's syndrome and pharyngotonsillitis in adolescents and young adults

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Background: *Fusobacterium necrophorum* (FN) has been known for several decades as the cause Lemierre's syndrome. Newer studies also show that FN is involved in peritonsillar abscess formation, otitis media and tonsillitis in adolescents and young adults. In addition, complications (peritonsillar abscesses and Lemierre's syndrome) due to FN tonsillitis appear to be more common than complications arising from *S. pyogenes* tonsillitis. In spite of the reoccurrence of this deadly bacterium, virtually nothing is known about the genomics, phylogeny, pathogenesis and virulence of FN. **Methods:** Nearly100 isolates of *FN* isolated from the Lemierre's syndrome patients, patients with tonsillitis, peritonsillar abscesses and other localized infections, healthy carriers and animals were included and sequenced with Illumina and PacBio. **Results:** Initial data from the genomes of 17 strains shows that human and animal strains are phylogenetic similar. In addition, invasive strains were found in two clonal lineages indicating clonal origin of invasive strains. The complete phylogenetic and comparative genomic analyses will be presented at ANNUAL CONFERENCE 2017. **Conclusions:** The results will greatly improve our general understanding of this emerging human pathogen and will be the basis for further studies on this deadly bacterium. Our ultimately goal from the analysis is to development of quick tests to detect FN directly from throat swabs similar to the Strep A test. The test will have the potential to greatly reduce complications such as peritonsillar abscesses and Lemierre's syndrome from an otherwise benign infection like tonsillitis.

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Community context - a main determinant for fitness of antimicrobial resistance

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To which extent and under which conditions antimicrobial resistance genes are selected for in gut microbial communities of farm animals is of acute relevance in the age of massive antibiotic usage. Here we are aiming at determining the effect of the community context on fitness and selection for a resistant mutant compared to its susceptible counterpart within a complex pig gut community and across an antibiotic gradient.

The focal species, red-fluorescently (mCherry) labelled E.coli, with and without an introduced antibiotic resistance gene was inoculated into anaerobic batch bioreactors containing artificial pig faeces medium. Competition experiments of resistant and susceptible focal species were carried out in presence and absence of a complex bacterial community extracted from fresh pig faeces across a gradient of Kanamycin ranging from minimum selective concentration (MSC) of the susceptible strain to extinction (MIC). Fitness and survival of the tagged focal species were monitored compared to the community as well as its susceptible counterpart.

Hosting Kanamycin resistance provided a minimal but significant advantage under non selective conditions, but a clear selective advantage at high antibiotic concentrations with and without the community present. Absence and presence of a competing complex community played a substantial role in selection for the resistant species as its fitness benefits were up to 10-fold ($p<0.05$) decreased in the selective window between MSC and MIC of the susceptible strain.

Our results suggest that determining selective conditions for resistant species have to be carried out in a complex community context rather than in single strain experiments.

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Fine stratification of microbial communities through a metagenomic profile of the Mediterranean photic zone

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Background
In the open sea, the differences of the microbiome between the photic and aphotic zone have been extensively illustrated and analyzed by multiple approaches. However, the photic zone, that is the most biologically relevant, is considered as a homogeneous single ecological compartment.
Methods
We took and sequenced samples from a depth profile (15, 30, 45, 60, 75, 90 and 1000m) at a single site in the off-shore Western Mediterranean. By high-throughput metagenomics, we were able to study the structure of the community, evaluate the presence of some ecologically relevant genes and reconstruct the genomes of representative microbes. Recruitment of all the assembled genomes plus some reference isolates was also carried out through the profile.
Results
Sequence similarity, clusters the samples by depth, with three main branches corresponding to upper photic (15 and 30m), deep chlorophyll maximum (45 and 60m) and lower photic (75 and 90m), 1000m appearing as an outgroup. We have retrieved 38 genomes belonging to the twelve predominant phyla and analyzed the precise depth distribution. The distribution of most of these microbes extends only over a 30 m thick layer within the ca. 100 m deep photic zone i.e. they are adapted to a narrow depth range. We also assembled and analyzed 168 rhodopsin molecules throughout the water column.
Conclusion
We have detected marked stratification at the level of large clades, but mostly at finer taxonomic levels (species), that reflect adaptation of microbial species to live at defined depth ranges in the water column.

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Comparative transportomics: definition of orthologous groups of transporters reveals highly dynamic evolutionary patterns of transporter use in the Enterobacteriaciae.

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Functional annotation of transporter genes in genome sequences is difficult and often poorly undertaken. Particularly for large gene families like the major facilitator superfamily (MFS) and ATP-binding cassette (ABC) transporters this task is compounded when annotation relies on annotation from the best-match of a BLAST analysis, obscuring the role of orthologs. Here we outline a simple new approach, comparative transportomics, that enables clear identification of direct orthologues, and hence proteins with likely similar substrate specificity, across bacterial genomes. We test this approach using a comparison of 14 strains of Salmonella enterica with the model organism E. coli K-12 for the MFS and ABC transporters in these genomes. This analysis shows that even for these two closely related genera, up to a third of the MFS and ABC transporters in these genomes are not orthologues across the two genera. For the MFS analysis, the approach confirms the lack of a lactose permease (LacY) orthologue, which agree with the Lac- phenotype of Salmonella sp., however, our results show absence, or non-orthologous gene-replacements, of orthologues of known E. coli transporters where Salmonella sp. can grow on the relevant carbon sources. This high-throughput approach further reveals gene duplication, loss or gain events in multiple transporters, where in most cases these events are exclusive to a smaller group of strains; perhaps due to shared ancestry/environment. Hence, we describe a powerful approach that leads to precise descriptions of orthologues in large gene families of transporters, a method that is generally applicable to comparison across many microbes.

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Characterisation of relatedness and antibiotic resistance patterns of *Escherichia coli* isolated from multiples sources in Nairobi, Kenya, by whole genome sequencing

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At present, transmission dynamics of resistant bacteria and their determinants between livestock and humans is poorly understood, particularly at the molecular level.

Using whole genome sequencing and bioinformatics analysis, our aim was to investigate patterns of genetic relatedness and to characterize antibiotic resistance profiles in *E. coli* isolates from 369 samples obtained from different sources/host populations in Nairobi, Kenya: humans, livestock, wildlife, food and the environment.

Our analysis identified 164 multi locus sequence types (STs), indicating a high clonal diversity within the *E. coli* population. The eight most common STs were distributed across multiple sources, indicative of frequent clonal spread. We found 34 antibiotic resistance genes (ARGs) across seven antibiotic classes. The great majority of common ARGs overlapped between humans and animals possibly reflecting overlapping antibiotic usage in human and veterinary medicine in Nairobi, or spill-over of resistance genes or bacteria between humans and animals.

This study demonstrated evidence of bacterial sharing and, importantly, overlapping patterns of STs and transferable ARGs, between humans and livestock in Nairobi.

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Selection for antibiotic resistance at very low antibiotic concentrations

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Antibiotic resistance is one the greatest threats to global health and the global economy. However, very little is understood about selection for antibiotic resistance, particularly at sub-therapeutic concentrations. Studies in recent years have shown that selection can in fact occur at extremely low antibiotic concentrations, though these have been largely limited to single host species competition experiments.
In this study, a single species competition experiment was compared to an experimental evolution microcosm experiment using a natural, complex community (untreated waste water). Both were exposed to a range of concentrations of the clinically important antibiotic cefotaxime and passaged daily into fresh media and fresh antibiotic for 7 - 8 days. qPCR determined prevalence of the worldwide pandemic CTX-M resistance gene group at the beginning and end of the experiments. Illumina MiSeq2 metagenomic sequencing for a subset of concentrations at the end of the complex community experiment was conducted to investigate possible co-selection for other resistance determinants, and any impacts on community structure.
Results to date demonstrate single species assays and complex communities produce different minimal selective concentrations. Selection was observed at very low antibiotic concentrations in the complex community, far below those used clinically, but this was not the case in the competition experiment. This suggests selection for CTX-M genes could occur in the natural environment where measured antibiotic concentrations are in the same order of magnitude; or even in the body during chemotherapy due to concentration gradients forming in different body compartments.

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Shark Vs. Tiger – Can experimental evolution be used to discover new antibiotics and improve existing antibiotic producing strains?

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Pseudomonas aeruginosa is a fast growing, opportunistic pathogen which is found in numerous environments including water and soil. It is well known as a multidrug resistant Gram-negative pathogen with a large number of virulence factors available to facilitate infection of the host. P. aeruginosa is also known to be highly adaptable and has previously been observed to undergo significant genomic changes under clinical, environmental and laboratory conditions. Streptomyces rimosus is a Gram-positive filamentous bacterium and prolific producer of the antibiotic oxytetracycline. Oxytetracycline is naturally produced by S. rimosus under conditions of environmental stress such as phosphate limitation. S. rimosus has previously undergone a traditional regimen of strain improvement using random mutagenesis resulting in strains that produce varying amounts of oxytetracycline from 1 g/l to >70 g/l. As with many other Streptomyces spp., the S. rimosus genome contains significantly more secondary metabolite biosynthetic gene clusters than the number of molecules that can be detected under laboratory conditions. This project aims to test the hypothesis that forcing P. aeruginosa and S. rimosus to compete for resources will provide the evolutionary pressure required to induce the production of novel secondary metabolites, enhance the production of known antibiotics or lead to increased resistance. A segregated co-culture system has been devised that enables the two organisms to compete for resources whilst allowing exposure to secondary metabolites produced by each organism. Co-culture experiments performed over a range of timescales will be presented along with a range of phenotypic assays.

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Environments mimicking periodontal pocket select pathogenic bacterial communities

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Background: Periodontal disease is dysbiosis that is a consequence of changes in resident oral microbiota and host responses to it. The "Ecological plaque hypothesis" argues that environmental changes in the periodontal pocket, which arise through increased inflammation, are associated with the enrichment of pathogenic bacteria.

Methods: Pooled saliva, supragingival and tongue plaque samples were inoculated into Calgary Biofilm Device in basal media ± serum to form biofilms and harvested after one and three weeks. DNA was isolated and metagenomes were sequenced on the Illumina HiSeq3000. Housekeeping 16S rRNA genes were extracted using HOMD (at 98,5% identity). Alpha diversity and weighted and unweighted UniFrac distances were compared using Kruskal-Wallis sum-rank test with *post hoc* analysis. Variance stabilizing transformation was applied to compare relative abundancies of species. The abundance of species was evaluated using DESeq2 package.

Results: Significant differences in species richness ($p<0.001$) and diversity ($p<0.001$) were detected comparing baseline, first and third week samples. Membership of communities was not significantly different ($p=0.06$), while structure of communities was significantly differed between some samples ($p=0.04$). Compared with baseline inoculum, biofilms grown in serum showed significant (adjusted $p<0.001$) enrichment of *Porphyromonas gingivalis, Parvimonas micra, Filifactor alosis, Tannerella forsythia*. *F. alocis, Dialister invisus, Treponema socranskii* (adjusted $p<0.01$) were enriched in biofilms grown in medium + serum compared with basal medium.

Conclusion: While richness and diversity decreased over culturing time, phylogenetic variation between communities was conserved. Biofilms cultured in serum-containing media became enriched with periodontitis-associated bacteria.

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The Population structure of non-typhoidal *Salmonella* in humans and animals in southern Vietnam

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Salmonella enterica is a species of Gram-negative bacteria that is able to infect and colonize a broad range of host species including mammals, birds, and reptiles. Non typhoidal *Salmonella* (NTS) refers to all *Salmonella* serotypes other than *S*. Typhi and *S*. Paratyphi A, which cause typhoid fever. NTS infections in humans is generally thought to be foodborne; however direct contact with infected animals and human to human transmission may also be another routes of transmission. We aimed to understand the population structure of NTS isolated across southern Vietnam. We hypothesized that human to human transmission may play a role in dissemination of specific *Salmonella* serotypes and genotypes. To test our hypothesis we isolated a total of 580 NTS isolates during a range of studies performed across Ho Chi Minh city and Mekong Delta from 2007 to 2013. We serogrouped all organisms using Multi locus sequence typing (MLST) by sequencing. Overall we identified 72 sequence types (STs) among 580 NTS isolates. We found that 9 STs were unique to animals, 29 STs were unique to humans and 34 STs were shared between human and animals. We found genetic discrepancy between NTS isolates that cause different disease syndromes in humans and also those that appear to have different tropisms to specific animals. This method is the first step to understand the diversity of NTS that cause different clinical manifestations in humans. Our data show that specific serotypes/sequences types and antimicrobial susceptibility profiles appear to have specific associations with animals and human disease.

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How does genetic background predetermine emergence of de novo resistance in *Staphylococcus aureus*?

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Understanding why some bacteria are extremely successful in evolving antibiotics resistance is crucial for developing treatment strategies to oppose global spread of resistance. Experimental evolution is a powerful tool for testing different aspect of microbial evolution, including antimicrobial resistance evolution.
In this study, we compare evolvability of more than 270 clinical isolates of *Staphylococcus aureus* towards clinically relevant concentrations of ciprofloxacin. Unlike many studies mainly focusing on intrinsic resistance in a small sample of isolates, we measure the evolutionary potential of the isolates with different genetic backgrounds which represent the global diversity of *S. aureus*. By simultaneously evolving hundreds of replicated populations under a controlled environment, we found that, despite sharing low levels of intrinsic resistance, the isolates exhibit large variation in evolving resistance. We confirmed that the resistance is typically gained via mutations in *grlA* and *gyrA* genes encoding the molecular targets of ciprofloxacin and other fluoroquinolones. However, our data suggest that the probability of those mutations to be acquired upon antibiotic treatment differs among isolates. By combining the results from this large-scale evolution experiment with whole genome sequence data from the isolates, we are currently investigating how genetic background predefines evolvability of resistance in *S. aureus* and which genetic loci are involved.
Identification of genetic factors which influence evolution of de novo resistance and understanding their molecular role should facilitate developing measures to stop emergence and spread of resistance.

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The spatiotemporal dynamics and phylogenetics of *Salmonella* Paratyphi A in Kathmandu, Nepal

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Enteric fever is a life threatening systemic disease caused by the bacteria *Salmonella* Typhi and *Salmonella* Paratyphi A, B, and C. *S.* Typhi is the major agent of enteric fever, yet *S.* Paratyphi A is emerging at an unprecedented rate in many developing countries such as South Asia and Southeast Asia. In our location in Patan Hospital, Kathmandu, standardized blood culture surveillance over the last ten years has shown annual increases in the proportions of individuals with *S.* Paratyphi A.There is no current vaccine against *S.* Paratyphi A and the licensed Vi-polysaccharide vaccines against Typhi do not provide cross-protection immunity against Paratyphi A infection and may become less useful in controlling enteric fever in regions where these bacteria are co-circulating.The increase in multidrug resistance and intermediate susceptibility to fluoroquinolone of *S*. Paratyphi A couples with a lack of accurate epidemiological data making this neglected pathogen an emerging global health issue. In this study, we performed whole-genome sequencing on 183 *S.* Paratyphi A isolates collected between 2005 and 2011 and combined these genomic data with individual GPS information to investigate the population structure and spatiotemporal dynamics of *S.* Paratyphi A in Kathmandu. We found that *S.* Paratyphi A has gone through a major clonal expansion in this location since 2005, mainly driven by resistance to fluoroquinolones. *S.* Paratyphi A isolates in Nepal have been introduced from other neighboring countries and replacing the native strains. Spatiotemporal mapping analysis demonstrated that unlike *S.* Typhi, *S.* Paratyphi A infections were more associated with outbreaks.

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Over-expression of antibiotic resistance genes in hospital effluents over time

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Background

Effluents contain a diverse abundance of antibiotic resistance genes that augment the resistome of receiving aquatic environments. However, uncertainty remains regarding their temporal persistence, transcription and response to anthropogenic factors, such as antibiotic usage.

We present a spatiotemporal study within a river catchment (River Cam, UK) that aims to determine the contribution of antibiotic resistance gene-containing effluents originating from sites of varying antibiotic usage to the receiving environment.

Methods

Gene abundance in effluents (municipal hospital and dairy farm) was compared against background samples of the receiving aquatic environment (i.e. the catchment source) to determine the resistome contribution of effluents. We used metagenomics and metatranscriptomics to correlate DNA and RNA abundance and identified differentially regulated gene transcripts.

Results

We found that mean antibiotic resistance gene and transcript abundances were correlated for both hospital (rho=0.9, two-tailed p<0.0001) and farm effluents (rho=0.5, two-tailed p<0.0001) and that two beta-lactam resistance genes (bla_{GES} and bla_{OXA}) were over-expressed in all hospital effluent samples. High beta-lactam resistance gene transcript abundance was related to hospital antibiotic usage over time and hospital effluents contained antibiotic residues.

Conclusion

We conclude that effluents contribute high levels of antibiotic resistance genes to the aquatic environment; these genes are expressed at significant levels and are likely related to the level of antibiotic usage at the effluent source.

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Evolution of a zoonotic pathogen: investigating prophage diversity in enterohaemorrhagic E. coli O157 by long-read sequencing

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Enterohaemorrhagic Escherichia Coli (EHEC) is a zoonotic pathogen known to be potentially lethal in humans. Its main animal reservoir is ruminants, specifically cattle, and yearly outbreaks occur worldwide with the most prevalent serotype being EHEC O157:H7. Most virulence factors of EHEC O157, including Shiga toxin (stx) genes, have been acquired through horizontal gene transfer, mostly as the integration of bacteriophages (prophages), which complicate genome assembly using the more prevalent short-read sequencing platforms. For our analysis selected representative strains from the EHEC O157 SNP phylogeny were sequenced using the long-read Pacific Biosciences platform. This has allowed an analysis of complete genomes from EHEC O157 strains, including the extraction, and comparison of their fully assembled prophage content. We observed different levels of variation across the prophage population which could be representative of the timing of acquisition events. Stx2c prophages exhibited minimal sequence variation compared to Stx2a- and Stx1a-encoding prophages, supporting the hypothesis that a Stx2c prophage acquisition event lead to the founder EHEC O157 clone. Acquisition of a specific type of Stx2a prophage into the Stx2c background resulted in conversion of the isolate from Phage Type (PT) 32 to PT21/28, the PT associated with the most serious EHEC O157 human infections in the UK over the last decade. By comparing the gene content of all prophages, it was evident that certain functional groups were over-represented or under-represented dependent on the size of the prophage. Recombinational activity was retained while phage structural genes were lost in the smaller and presumably cryptic prophage regions.

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Investigating the phenotypic and genotypic diversity of *Helicobacter pylori* populations within different niches of the human stomach

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Helicobacter pylori infection usually results in asymptomatic gastritis but 10%-15% of infected people develop peptic ulceration and 1%-3% develop gastric adenocarcinoma. Infection usually persists lifelong. High mutation and recombination rates are thought to aid in host adaptation and persistence of infection, but phenotypic and genotypic diversity of *H. pylori* populations between different niches of the same stomach is not yet well understood. We aimed to compare *H. pylori* populations from the corpus and antrum regions of the stomach to determine whether the harsher environment of the corpus (which contains acid-producing cells) drives increased population diversity.

We investigated the phenotypic and genotypic diversity of *H. pylori* sweeps cultured from the gastric biopsies of patients attending the Queens Medical Centre in Nottingham. Paired biopsies from the antrum and corpus regions were obtained from 9 patients; single biopsies from one region were obtained from 18 patients. Antibiotic resistance and superoxide assays were used to investigate the potential effects these varying niches might have on bacterial phenotype and virulence. Populations from the corpus had higher levels of resistance to one or more antibiotics compared with the paired antral populations, but no differences were observed for superoxide resistance.

Additionally, we now propose a novel approach to *H. pylori* whole genome sequencing by utilising deep sequencing to analyse minor allele variants within clinical sweep populations. Our ongoing genomic analyses aim to improve understanding of how diversity of *H. pylori* populations varies between different niches of the stomach.

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Resistomics of sputum samples from chronic obstructive pulmonary disease

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The prevalence of antimicrobial resistance (AMR) genes is a major global threat. While the human gut microbiota has been extensively studied, little is known about the bacterial community in the lower respiratory tract as a reservoir and source of AMR genes. Chronic Obstructive Pulmonary Disease (COPD) is a common respiratory condition associated exacerbations that are usually treated with antibiotics. We studied the prevalence of AMR genes in COPD sputum samples.

Using high-throughput quantitative PCR targeting 296 specific AMRs, we determined the prevalence of AMR genes in DNA extracts from 165 well-characterized sputum samples from patients with COPD. We previously determined the bacterial microbiomes in these samples. Of the 245 AMR genes detected, antibiotic deactivation and efflux pumps were the dominant mechanisms. The 10 most prevalent AMR genes detected were *mefA*, *matA/mel, sulA/flop* and *tetM* (>90%), *cfxA* and *pmrA* (>80%), *tetQ, fabK, ermB* and *IS613*(>60%). In addition signals indicating the presence of extended spectrum beta-lactamases and carbapenemases were also obtained and analyses to confirm these observations are in progress.

We note that phenotypic resistance was rarely detected by routine clinical analyses and speculate that genes carried by organisms that are not recognized as pathogenic may nonetheless affect responses to therapy and contribute to the spread of AMR.

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Population genomics of Legionellosis linked to growing media samples reveals hidden complexities of source attribution

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Legionella longbeachae causes the majority of legionellosis cases in Australasia and some Asiatic countries and is an emerging pathogen in Europe and USA. However, our understanding of *L. longbeachae* genetic diversity and population structure is very limited. Here we carry out a genomic epidemiological analysis of *L. longbeachae* isolates from a cluster of legionellosis cases in Scotland in 2013 linked to commercial growing media, in comparison to isolates from diverse patient and environmental sources in different countries. We identified extensive genetic diversity across the *L. longbeachae* species due in part to intra- and interspecies recombination, and a wide distribution of closely-related genotypes consistent with global routes of dissemination. Importantly, we observed a diverse pool of *L. longbeachae* genotypes existing within compost samples which may limit the potential for source attribution. These data represent the first view of the genetic diversity of *L. longbeachae* populations providing key information for future investigations of outbreaks caused by this emerging pathogen.

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Linking genomic variation to transcriptomic variation within Rhizobium leguminosarum

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Rhizobium leguminosarum biovar trifolii (Rlt) forms a symbiotic relationship of agronomic importance with white clover (Trifolium repens). The symbiosis enables increased plant growth by fixing atmospheric nitrogen, providing an alternative, sustainable fertilization method over exogenous commercially-available nitrogen fertilizer. On organic farms and areas with reduced biologically available nitrogen the symbiosis is vital to crop productivity. Rlt strain isolates display wide genomic variation, especially with regards to accessory genome content. Our genomic analyses show Rlt strains can be categorized into five genospecies, based on their core genomes. It is unclear whether this genomic variation influences transcriptomic variation. Furthermore, the extent to which differential gene expression profiles correlate to variation in symbiotic capabilities is unknown. To date, no studies have compared transcriptomes of different Rlt genotypes, or transcriptomes of the same strain over a large geographical range. We aim to investigate this by undertaking whole-genome transcriptome analysis of 100 genome-sequenced Rlt isolates obtained from clover nodules across Europe by the NCHAIN project. We demonstrate large reproducible variations in yield are obtained when the same clover genotype is inoculated with different Rlt strain isolates. Similarly, different clover genotypes display varied responses to inoculation of the same Rlt strain. Therefore, symbiotic efficiencies are potentially affected by Rlt strain and clover genotype specificity. We suggest clover yield can be increased by carefully complementing specific Rlt genotypes to clover genotypes. Ongoing transcriptomics work will focus on linking genotypic variation and symbiotic efficiency at the gene expression level, to elucidate the symbiosis at a more mechanistic level.

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Genomic investigation of a recurrent *Escherichia coli* ST131 infection in a single individual over a five year period

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Recurrent urinary tract infections (rUTI) are extremely common, with ~25% of all women who suffer an initial UTI experiencing a recurrence within one year of the original infection. rUTI often occurs when bacteria from the initial infection persist after treatment. Here we use whole genome sequencing to examine the stability and dynamics of an *E. coli* ST131 population isolated over a 5-year period from the urine and feces of a single individual (patient #1), and elderly women with rUTI since the 1970s.
67 ST131 isolates were collected from patient #1 between 2012 and 2016 and sequenced on the Illumina platform. Four isolates from patient #1, one from each of the years 2012 to 2015, were further sequenced using PacBio SMRT sequencing to produce complete reference genomes, allowing for the accurate profiling of insertion sequences and plasmids.
Here we found that ST131 urine and fecal isolates from patient #1 are highly similar. In contrast, ST131 strains isolated from other patients in the same region revealed a diverse population that spanned the previously defined clade structure of this clone. Our data also reveal a remarkably high level of plasmid diversity within ST131 isolates from patient #1, characterised by instances of plasmid loss and transfer of a large multidrug resistance island between plasmids. Furthermore we observed the emergence of a hetero-resistant population, three years after initial sampling, and show that both gentamicin resistant and susceptible ST131 variants persist in the fecal flora, providing compelling evidence of an intestinal reservoir as a source of rUTI.

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Exploitation of random transposon mutagenesis data to reveal unannotated genes and anti-sense features

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

A mini-*Tn*5 transposon library was constructed in *E. coli* K-12 strain BW25113. The transposon library was sequenced by following an amended TraDIS protocol. A total of 904,084 unique transposon insertion points were identified in order to establish the essential genome of *E. coli* K-12.

Outward reading transcription from a transposon is designed to avoid polarity artefacts, but a consequence of this is orientation-dependent insertion bias. Our detailed analysis of the orientation of insertions within the *E. coli* K-12 transposon library has revealed previously unannotated features. We will demonstrate that unique transposon insertion profiles can reveal unannotated genes upstream of an essential gene, the transcription start site location of an essential gene and, most significantly, the presence of anti-sense features opposing essential genes.

Our findings have potentially revealed previously unannotated features within the highly studied *E. coli* K-12 genome. In addition, recognition of these insertion profiles in transposon mutagenesis datasets would assist genome annotation of less well characterised genomes.

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Genetic diversity in modern lineages of *M. tuberculosis* indicates specialisation into hyper-virulent niche

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Background: The extremely low sequence diversity of Mycobacterium tuberculosis today can be fully explored with whole genome sequencing, allowing for genetic diversity to be explored across the species's multiple phylogenetic lineages.

Material/methods: 7,656 genomes of *M. tuberculosis* were assembled into draft genomes with Velvet, and scanned by BIGSDB for matches to genes in H37Rv. Isolates were assigned to seven known phylogenetic lineages (Comas 2013, Nature Genetics) by a clustering algorithm. Genes were assesed for their allelic diversity (unique sequences at each locus, divided by their length) and selection strength via the relative abundance of non-synonymous mutations (ratio of unique sequences before and after translation).

Results: The “Modern MTBC” lineages (L2, L3 and L4) differed from the background levels of genetic diversity in some functional categories. Genes in the “Internal metabolism” and “Regulatory mechanisms” had especially conserved sequences, while the “Cell Wall” category had higher diversity than expected Genes in the “Virulence” category are under heightened positive selection in the modern lineages, further supporting the hypothesis that these lineages are further specialised into hyper-virulence. This is consistent with a pathogen that is further specialising to a metabolic niche, which adapting to a new immunological landscape by changing its outer membrane and exuded proteins.

Conclusions: “Modern lineages” of *M. tuberculosis* have conserved metabolic loci, indicating further specialisation, matched with increased diversity in excreted proteins, indicating adaptation to immune system response. This is consistent with further specialisation into a hyper-virulent niche, perhaps explaining the disproportionaly high number of cases caused by these lineages.

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Understanding within patient variation of Uropathogenic *Escherichia coli* Ruqaiah Ismael Bedaiwi[1], Mathew Diggle,[1]*Dr.Alan McNally*

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Urinary tract infections (UTI) are the most common infections worldwide. UTI are most commonly caused by Uropathogenic *E. coli* (UPEC). UTI are routinely diagnosed and treated based on clinical bacteriology performed on a single colony. However, recent work on infections such as cystic fibrosis has highlighted the presence of multiple phenotypic and genotypic variants within a single infected patient. Here we present work investigating the level of within-patient diversity of UPEC at a phenotypic and genotypic level. Forty-two urine samples were collected and antibiotic sensitivity testing performed on each well-isolated colony. Samples are classified based on their sensitivity patterns into three groups: identical phenotypes, low diversity phenotypes and high diversity phenotypes. Nine urine samples were categorized as high diversity phenotypes. Phenotypic assays of high diversity samples show some variation in motility, biofilm formation and association and invasion assays. We also present genome-sequencing data correlating the levels of phenotypic diversity with genetic changes. Together our data is the most high-resolution snapshot to date of the levels of extant diversity of UPEC within patients with acute UTI.

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FNR is a regulator of SPI-2 in *Salmonella* Typhimurium

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Salmonella enterica serovar Typhimurium is a Gram-negative facultative intracellular pathogen and a major cause of bacterial enteric illness in both humans and animals. The infection process of *S.* Typhimurium is made possible by the expression of two virulence-associated type three secretion systems that are encoded within the *Salmonella* pathogenicity islands 1 and 2 (SPI-1 and SPI-2). The regulation of SPI-1 and SPI-2 is complex and involves multiple transcription factors that ensure correct spatio-temporal expression during infection. *S.* Typhimurium expresses SPI-1 in the gut lumen which facilitates epithelial cell invasion and relies on SPI-2 expression for survival and proliferation inside epithelial cells and macrophages. However, studies have shown that SPI-2 is induced by an unknown signal prior to the invasion of epithelial cells in the gut lumen. It is becoming increasingly recognized that enteric pathogens including *S*. Typhimurium use O_2 as a signaling molecule, triggering expression of virulence traits. We are investigating the regulation of SPI-2 by environmental signals, such as oxygen and during growth with alternative electron acceptors. We found that SPI-2 regulation is tightly controlled by a transcription factor under static growth in a minimal medium supplemented with the alternative electron acceptors fumarate and TMAO.

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Host proteome study during urinary tract infections

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Urinary tract infections (UTIs) are one of the most common infections globally. They affect nearly half of all women, and are mostly caused by uropathogenic Escherichia coli (UPEC). A proportion of them will experience recurrent infections (rUTIs) within a year, often with the same bacterial strain. While antibiotic therapy is generally effective, rUTIs are a chronic and debilitating problem. Both mouse and cell line models of infection have given us insights into specific host pathways and UPEC virulence mechanisms involved in the invasion process. The former has suggested that intracellular infection by UPEC contributes to recurrent disease.
We have developed technology for isolation of single mouse epithelial cells that could enable unbiased approaches to understanding intracellular infection stages. While these isolated cells are being used for genomics, our work explores the possibility of performing a proteomic analysis on them. This may allow for a better understanding of host response pathways during infection. The primary technical issue is sensitivity for single epithelial cells. Using a mass-spectrometry based approach, we successfully identified a total of 2398 proteins. Overall, we found high similarity host protein profiles between infected and uninfected epithelial cells. We also find significant changes in the abundances of four proteins, all of which have not been previously implicated in infection. We are currently validating these results, which could have great potential for additional human studies and development of therapeutic targets to control recurrent UTIs.

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Characterisation of genes identified by TraDIS as being involved in phage susceptibility in *Escherichia coli* O157:H7

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Phage typing has traditionally been used to study the epidemiology of particular bacterial infections, including *E. coli* O157. Certain phage types (PTs) are more likely to be associated with human infection and so understanding the genetic basis to typing may provide insights into strain pathogenesis as well as providing further basic information on bacterial sensitivity to lytic phages which may have value for predictive phage therapy. In this study, TraDIS, a genome-wide mutagenesis method, was used to screen for genes involved in phage susceptibility using a specific *E. coli* O157 strain.

A transposon mutant library was created and TraDIS specific sequencing indicated high-density transposon insertion sites throughout the genome (~1 insert per 50 bp). The library was then exposed to a fully lytic phage selected from the typing panel and mapped read density compared for phage exposed and control populations. A change in the number of reads indicates altered sensitivity. 115 genes were associated with phage sensitivity and 44 genes with phage resistance. As anticipated, as a key receptor, insertion in *ompC* and its regulators *ompR* and *envZ*, significantly reduced phage sensitivity. It was noted that insertions in genes of the *sap* operon also increased phage resistance a finding at odds to a protective role in relation to specific antibiotics. Insertion in *sspA* (stringent starvation protein) had the clearest effect on increasing phage sensitivity and the mechanism for this is currently under investigation along with genes identified that appear not to be linked to established bacteriophage infection pathways.

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Comparison of the Vaginal and Intra-amniotic Microbiome of Women giving Birth Preterm

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Background: Few comparisons have been made between the vaginal and intra-amniotic microbiota of women giving birth preterm. To understand the relationships between both genital microbiomes, their compositions in women giving birth preterm by Caesarean section were investigated.

Methods: Genital swabs were obtained from 50 mestizo women from Northern Peru; gestational ages at birth were 31 preterm, 9 very preterm and 10 extremely preterm. Twenty-seven participants had infections during pregnancy. Participating women were administered a questionnaire including demographic data and summarising the history of the pregnancy related to infection and other complications. DNA was extracted from swabs taken from the upper vagina and three intra-amniotic (amniotic fluid, surface of the placenta and axilla of infant), and the identity of the taxa present was determined by ultrafast sequencing of the V1V3 regions of the 16S rDNA gene of samples. The sequence data were analysed with MOTHUR and statistical analyses were performed employing various bioinformatics tools.

Results and Discussion: The relative abundances of some phyla were different in the vaginal and intra-amniotic microbiomes. At the genus level, differences in bacterial community structures were observed between the three intra-amniotic sites. There were no significant differences between the microbiomes of women giving birth at different various ages. PERMANOVA analyses yielded significant differences between the genital microbiomes of women with infection during pregnancy and those without complications. The presence of some genera in the genital microbiomes were associated with a history of infection.

Annual Conference 2017
EICC, Edinburgh
3-6 April

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Conservation of amylosome organization inferred from genomic analysis of *Ruminococcus bromii* strains from the rumen and human colon.

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Whole Genome Sequencing to Predict Antimicrobial Resistance in *Salmonella enterica*

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Whole genome sequencing (WGS) has been performed on all presumptive isolates of *Salmonella* received by the reference laboratory at Public Health England (PHE) since April 2014 and has proven to be an invaluable addition to the surveillance of *Salmonellosis,* offering unprecedented precision in linking cases of infection. WGS also provides the opportunity to characterise phenotype-to-genotype relationships such as the resistance to antimicrobials.

Here we used 'Genefinder', a bioinformatics pipeline developed by PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, to predict antimicrobial susceptibility of isolates from genotype. This pipeline was validated by assessing correlation between genotype and phenotype for >5200 *S. enterica* isolates (246 serovars). Clinical MICs were determined for 16 antibiotics (Gram negative panel for Enterobacteriaceae and azithromycin) using EUCAST methodology and breakpoints.

'Genefinder' is a customized algorithm that uses Bowtie to map reads to a set of >2500 reference sequences. The data are parsed based on read coverage (100%), base-call variation (85%) and nucleotide identity (90%) to determine the presence of the reference sequence or nucleotide variation within that sequence. Known acquired resistance genes and/or resistance-conferring mutations relevant to β-lactams, fluoroquinolones, aminoglycosides, chloramphenicol, macrolides, sulphonamides, tetracyclines, trimethoprim, rifamycins and fosfomycin were included in the analysis.

Specificity and sensitivity of prediction of phenotype from genotype exceeded 99.2% for all antibiotics tested apart from ciprofloxacin (0.5 mg/L) (sensitivity: 97.1%), and streptomycin (sensitivity: 98.8%, specificity: 97.4%). The high sensitivity and specificity indicated suitability of the pipeline to predict AMR phenotype from genotype for *S. enterica* isolates.

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Glycomacropeptide sustains microbiota diversity and promotes specific taxa in an artificial colon model of elderly gut microbiota

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Background: Recent studies have suggested a strong diet -gut microbiota- health link. Here, the potential of milk-derived glycomacropeptide (GMP) and lactose for modulating the human gut microbiota of older donors, in whom loss of diversity correlates with inferior health was investigated.

Methods & Results: We used an *in vitro* batch fermentation model to simulate colonic fermentation processes of two GMP products, i.e. a commercially available GMP concentrate (CGMP-10) and a semi-purified GMP concentrate (tGMP), and lactose. Glucose-supplemented and carbohydrate-depleted medium were used as controls. Faecal samples included those from healthy and frail older subjects. Samples were analysed by Illumina Miseq sequencing of rRNA gene amplicons. CGMP-10 had a positive effect on the growth of health-related taxa like *Coprococcus* and *Clostridium* cluster XIVb. GMP-containing media sustained higher faecal microbiota diversity after 24 hrs of fermentation compared to control substrates or lactose. Lactose fermentation promoted the growth of *Bifidobacterium* but also of Proteobacteria including Escherichia/Shigella.

Conclusion: This work provides an in-depth insight on the potential of GMP and lactose for modulating the gut microbiota and contributes more evidence confirming the prebiotic activity of GMP.

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Whole genome taxonomic reclassification and a universal prokaryotic identifier scheme

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Tradtional species concepts are problematic for prokaryotes, but taxonomic classification by 'species' remains critical for development of practical policies, legislation and diagnostics that aim to reduce risk from pathogenic bacteria. We present a novel, robust, graph-based whole genome classification method that produces stable relational classifications for prokaryotes, retaining familiar features that provide continuity with existing nomenclature.

Average Nucleotide Identity (ANI) calculates a matrix of distance measures for any set of prokaryotic genomes. Modular graph decomposition of this matrix into cliques identifies self-describing subdivisions analogous to existing classifications (genus, species, etc.), and novel interstitial groups. These cliques require no arbitrary thresholding, and are stable to addition of new sequences. The groupings are uniquely and stably identified, providing bases for universal indexing of prokaryotes, and probabilistic estimates of risk phenotypes conditioned on clique membership.

We demonstrate software implementation and application of this method to pathogenic and industrially-useful bacteria, showing that continuity of existing nomenclature is maintained while enabling richer, stable classification independent of species concepts. We pay particular attention to soft-rot enterobacterial plant pathogens, proposing useful reclassifications at genus, species, and subspecies levels. We also apply this technique to Pseudomonas, Streptomyces, Escherichia coli, and other human pathogens, with proposals for reclassification.

Using whole genome analyses, an unambiguous and extensible classification system is created, retrospectively mapping to existing nomenclature and applicable to existing databases, but independent of historical phenotypic and morphological species concepts. This classification approach may provide novel bases for regulatory agency policy and legislation, and a universal prokaryotic identifier scheme.

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Not so plastic after all: genomics of Acinetobacter baumannii pulsotype SMAL

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Background: Acinetobacter baumannii SMAL is a pulsotype circulating in Italy since 2002. SMAL isolates are characterized by strong biofilm formation, and by variable antibiotic resistance profiles, with a 37% prevalence of carbapenem resistance. SMAL has been epidemiologically classified as a low incidence but high persistence pulsotype, that has plagued Italian hospitals for the last 15 years.

Methods: Fifteen genomes of SMAL strains isolated from 2004 to 2011 were sequenced and compared with the publicly available A. baumannii genomes using SNP-based phylogeny. Analyses of gene content, recombination and presence of insertion sequences (IS) were also run. Plasmidic sequences were assembled and manually closed.

Results: Three SMAL isolates presented a plasmid carrying the blaOXA-58 gene, while blaOXA-23 was found chromosomally encoded by two strains. The SMAL isolates resulted closely related to three American genomes. Gene content and recombination analyses showed very low genome plasticity in the SMAL cluster and high similarity with the American strains. SMAL and American genomes had a strikingly high ISs content. One sequence of class IS66 was found to inactivate comEC/Rec2, in all the Italian genomes, but not in the American ones. This gene is responsible for the uptake of exogenous DNA.

Conclusions: SMAL was introduced in Italy in one single event. The evolution of SMAL is marked by ISs proliferation, which by inactivating comEC/Rec2 probably reduced the capacity of the strain to recombine and acquire novel genes. We postulate that this evolutionary trajectory resulted in the current low proliferating, yet successful A. baumannii strain.

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CRISPR-associated formation of clonal lineages in a global population of Yersinia pseudotuberculosis

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Our knowledge of how bacterial pathogens have evolved is currently hampered by inadequate information on the full ecology of these organisms. In this work, we present data on population genomic analysis of Yersinia pseudotuberculosis, an important human intestinal pathogen, and for decades, a model organism in the study of microbial pathogenesis and evolution of mammalian virulence. The genomes of 134 Y. pseudotuberculosis isolates were sequenced and their phylogenetic relationships were determined based on core genome single-nucleotide polymorphism analysis, and compared to the grouping of the strains based on various datasets, which include clustered regularly interspaced short palindromic repeat (CRISPR) spacer distributions. We combined information on isolation, phylogeny, core genome recombination, and CRISPR arrays to investigate whether the ecology of the Y. pseudotuberculosis population overlaps with genetic patterns. There is little known regarding the phylogenetic distribution and potential role of CRISPR-Cas systems in modelling the accessory genome. Recent work on Yersinia enterocolitica suggested that distinct phylogroups of the species may be ecologically separated, through an exhibition of restricted genetic exchange between phylogroups. This draws parallels with our analysis, which reveals a globally dispersed population of Y. pseudotuberculosis that has diverged into subgroups of phylogenetically distinct lineages with associated CRISPR cassettes and limited levels of detectable shared recombination events between CRISPR clusters. Our analysis suggests that these CRISPR clades differ in the combination of genes that they carry, rather than individual genes, and their accessory genomes have been “locked” for a long time, thus resulting in the independent evolution of these lineages.

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Genome sequence analysis of four non-pathogenic *Neisseria* isolates reveals virulence genes.

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The human pathogen *Neisseria meningitidis* shares a niche in the human body with other bacteria of the same genus. Due to the natural competence for transformation of the species within *Neisseriaceae*, these commensal non-pathogenic *Neisseria* spp. represent a significant pool of additional genetic material from which *N. meningitidis* can draw. Here are presented data from the genome sequences of four isolates taken from the throats of two human volunteers, each representing a genetically distinct *Neisseria* spp. Amongst the genes present in these genomes are those that are generally associated with the pathogenic *N. meningitidis* and *Neisseria gonorrhoeae* and are considered virulence genes. Included in these are capsule-related genes, which may perhaps provide an additional genetic reservoir for capsule switching in the meningococcus. Horizontal gene transfer from non-pathogen to pathogen has been demonstrated previously, with this study providing further support for a pan-genome for *N. meningitidis* that includes all of the *Neisseriaceae*.

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Application of massively-parallel sequencing to investigate the dynamics and genetic basis of systemic salmonellosis in cattle

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Sequencing technologies have transformed our understanding of bacterial pathogenesis. Here we describe the use of sequencing-based approaches to study the dynamics and genetic basis of *Salmonella* pathogenesis in cattle. *Salmonella* serovars can produce a spectrum of illness in cattle from subacute infections and enteritis to typhoid-like disease, thus exerting a significant burden on bovine welfare and productivity. Human non-typhoidal salmonellosis occurs through consumption of ground beef, in particular owing to contamination from peripheral lymph nodes. Existing vaccines confer limited serovar-specific protection. Thus, understanding the lymphatic survival of *Salmonella* is key to developing effective vaccines and intervention strategies. Our research aims firstly, to identify serovars that are better adapted to survival within the bovine lymphatics, and secondly, to find *Salmonella* genes that aid this survival. Using massively-parallel whole-genome sequencing and by quantifying inter-serovar nucleotide differences, we can determine the relative abundance of multiple serovars during mixed infections. By applying this to mixed populations of serovars recovered from infected bovine tissues, including the gut and peripheral lymph nodes, we were able to define the dynamics of serovar spread and survival. Further, using massively-parallel sequencing in transposon-directed insertion-site sequencing (TraDIS) we can simultaneously assign identity and phenotype to bacterial mutants during infection. We previously assigned roles for 2721 *S*. Typhimurium genes in intestinal colonisation in cattle. Retrospective analysis of the same mutant library but recovered from mesenteric lymph nodes draining the terminal ileum in the same animals has identified genes with a putative role in lymphatic spread and/or survival.

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Characterising the phasome of carriage and disease isolates of *Haemophilus*

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Phase variation (PV) is a reversible switching of gene expression from an 'ON' to an 'OFF' state, commonly mediated by slipped strand mismatches at simple sequence repeats (SSRs) during replication (Henderson *et al.* 1999). *Haemophilus influenzae* - a common cause of paediatric otitis media - utilises PV to switch expression of factors involved in pathogenesis, including genes involved in modification of the LOS (Fox *et al.* 2014). The presence of phase variable genes can be inferred by identification of a cognate SSR.

Here we have used Phasome*It*, a novel program identifying putative phase variable genes from Next Generation Sequence data, to analyse all publically available *Haemophilus* genomes, in addition to a further 96 non-typable *Haemophilus influenzae* (NTHi) genomes generated by De Chiara *et al* (2014).

We have identified 333 discreet, putative PV loci, with an average of 24 loci per genome. The commensal species included in the analysis were shown to contain a lower number of PV genes (~8), compared with *H. influenzae* (26) and the pig pathogen *H. parasuis (28),* indicating diverse selective pressures experienced by pathogens. Conservation of phase variability was identified in *H. influenzae* mainly pertaining to genes which modify the LOS, and those involved in iron acquisition, implying an essential role for variation of their expression during carriage and disease.

Additional outputs of Phasome*It,* including correlation of ON/OFF expression states with disease source and differences in SSR tract length will be discussed.

Microbial Physiology, Metabolism and Molecular Mechanisms Forum

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Mechanistic and Phenotypic Characterisation of Rgg/SHP Quorum Sensing System in *Streptococcus pneumoniae* .Iman Abdullah, Russell Wallis, Peter Andrew and Hasan Yesilkaya. Department of Infection, Immunity and Inflammation, University of Leicester, UK

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Background: The Rgg regulators with their signaling peptides SHP (short hydrophobic peptides) form part of a quorum sensing system in Gram positive bacteria. They play an important role in stress response, sugar metabolism and virulence, but their functions remain unclear in *Streptococcus pneumoniae.* The pneumococcal D39 has five homologues of Rggs, and two of them (Rgg144 and Rgg939) are predicted to be associated with SHP peptides, which regulates its own expression, and is predicted to be required for Rgg activation. Therefore, this study was designed to characterise the importance of SHP144 residues in Rgg144 activation, binding, and phenotypic manifestation.

Methods: Several synthetic peptides representing C-terminal end of SHP144 were synthesised to identify active SHP144 using a promotor reporter essay. Site directed mutagenesis was used for substitution of selected residues of SHP144 with alanine, and effect of each amino acid replacement was studied using reporter assay. The phenotypic impact of mutations were determined by H_2O_2 resistance, and by growth assays in chemically defined medium supplemented with different sugars.

Results: The results showed that 13-aa long synthetic peptide representing C-terminal end of SHP144 was sufficient to stimulate Pshp144 in wild type and Δ*shp144* background. More interestingly, replacement of majority of selected residues with alanine abolished *shp144* transcription. In addition, mutations of selected SHP144 residues decreased pneumococcal resistance to H_2O_2, and diminished its growth in CDM supplemented with mannose.

Conclusion: The SHP144 peptide regulates its own expression. The composition and size of SHP144 play a significant role in Pshp144 transcription, and Rgg144's function.

Microbial Physiology, Metabolism and Molecular Mechanisms Forum

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The effect of hydrogen peroxide on the ferrous iron transport systems EfeUOB and FeoABC in *Escherichia coli*

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Iron is an essential element for bacterial growth. However, it is a dangerous metal because it has the ability to catalyse reactive oxygen species (ROS) through the Fenton reaction. The oxidation status of iron in the environment is largely determined by the pH and oxygen levels, with the poorly soluble ferric (Fe^{3+}) form persisting with high pH and O_2, and the more soluble ferrous form (Fe^{2+}) favoured by low pH and O_2. This study explores the differences in the activities of the two ferrous-iron transporters (Feo and Efe) of *E. coli* in their responses to hydrogen peroxide. *E. coli* mutants devoid of iron-transport systems were employed along with low-copy number plasmids or inducible plasmids (pBAD) carrying either *efeUOB* or *feoABC*. Results showed that H_2O_2 enhanced ^{55}Fe uptake for *efeUOB* transformants whereas provision of exogenous catalase caused strong inhibition. In contrast, FeoABC dependent iron-uptake was enhanced by catalase but inhibited by H_2O_2. This finding matches the predicted role of EfeUOB as a haem-peroxidase-dependent ferrous-iron transporter and suggests that Feo activity is subject to peroxide inhibition, possibly to limit iron uptake during redox stress. Thus, Feo and Efe provide alternative routes for ferrous iron transport in response to H_2O_2 availability, Feo being shut down by peroxide and Efe being activated.

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Inducing bacterial suicide as a new strategy to treat infections

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Antibiotic-resistant bacteria that cause infections that are difficult to treat are a serious and growing health threat. There is an urgent need for alternative antimicrobials to combat resistant infections. Toxin-antitoxin (TA) systems are natural molecular time-bombs that are encoded by the genomes of most bacteria, including human pathogens. TA cassettes consist of a pair of genes that encode for a toxin protein and its cognate antitoxin molecule. Under stressed conditions, bacteria activate the toxins components from TA complexes to cause reversible damage from within the cell that slows down bacterial metabolism. This study uses YefM-YoeB of *Escherichia coli* as an example TA to examine the mechanism by which the YoeB protein toxin is activated and the interaction of the toxin with the YefM antitoxin protein. The effects of random pentapeptide insertions on the toxic activity of YoeB were assessed. Pentapeptide insertions introduced into secondary structure elements generally abolished toxin activity. However, insertions that were located in loop regions of the toxin resulted in neutral effects on YoeB activity. The effects of the collection of pentapeptide insertions in YoeB on the interaction with YefM also were examined. The study provides valuable information on the functional regions of the YoeB toxin that are tolerant and intolerant of insertions. These insights will be useful in designing small molecules that disrupt the YoeB-YefM complex thereby releasing the toxin artificially and promoting bacterial suicide.

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Epstein-Barr Virus nuclear antigen-1, action and reaction as an oncogene

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The herpesvirus Epstein-Barr virus (EBV) leads to a life-long persistent infection and greater than 90% of the adult population of the world are seropositive. EBV is the causative agent of infectious mononucleosis, but in addition, the virus is associated with certain tumours of B-cell and epithelia cell origin (as well as some rare T-cell tumours). EBV nuclear antigen 1 (EBNA1) is an essential viral protein, required for the maintenance, replication and mitotic segregation of viral genome episomes. EBNA1 is a DNA binding protein and also interacts with several cellular proteins, thereby affecting host cell-signalling pathways and in so doing may contribute to cell survival and proliferation. EBNA1 is the only latent protein that is expressed in all EBV-associated malignancies and is thought to play a significant role in viral tumourigenesis.

In order to explore the oncogenic mechanism of EBNA1, B-cell lymphoma samples from transgenic mice expressing EBNA1 (EμEBNA1 mice) and/or c-Myc (Eμc-Myc mice) were analysed by immunoblotting. Several candidate cellular proteins likely involved in the tumourigenic process were examined, including C-myc, Mdm2, p53, PTEN, Akt and others.

Overexpression of specific MDM2 isoforms were detected in all EBNA1 tumour samples, not detected c-Myc tumour samples, or in pre-tumour or transgene negative samples. Thus there is a specific correlation of the overexpression of Mdm2, with EBNA1-induced tumourigenesis, likely reflecting the underlying mechanism. In addition, C-Myc was overexpressed in EBNA1 tumours, supporting our previous observations regarding the cooperation of these two proteins in tumourigenesis.

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Synergistic and antagonistic effects of copper and titanium on antimicrobial resistance in novel Paenibacillusspp isolated from historically metal-polluted urban soils James Delaney, Alyaa Abdelhameed, Matthew Hitchings, Ricardo del Sol and Geertje van Keulen Institute of Life Science, Medical School Swansea University, Swansea, Wales, UK

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Due to rapid industrialization and uncontrolled urbanization, soil contamination with heavy metals is a historical and continuing widespread problem, especially prominent in the vicinity of mines, smelters and industrial facilities. More recently, a correlation was determined between recent metal pollution and antimicrobial resistance (AMR) in agricultural and waste water treatment settings through cross-, or co-resistance to metals.

The objective of this study was to determine the levels of antimicrobial resistance and metal tolerance in novel Paenibacilli from historically polluted environments, to provide data to assess the risk of environmental AMR to public health from (archived) polluted soils by analysing a relatively understudied genus.

Draft genome sequences of seven environmental isolates of Paenibacillus showed the isolates may form new species within the genus. Maximum tolerance concentrations (MTC) were determined for two metals (Cu(II) and Ti(III)) present at high concentrations in the isolates' original habitats after which Minimum Inhibitory Concentrations (MIC) were determined for three common antibiotics (tetracyclin, cephalexin, and ciprofloxacin) in the absence or presence of metal at MTC.

Bacillus cereus ATCC14579^{T} (Firmicute control strain) showed MTCs for Cu(II) and Ti(III) of 100 µM and 200 µM, while MTCs for Cu(II) and Ti(III) varied from 20-100µM and 200-1000 µM, respectively for the seven novel isolates. The presence of metals at MTC affected MICs dramatically, with isolates displaying either increased antibiotic susceptibility or resistance, particularly with cephalexin. Comparative genomics studies are currently being undertaken to explain the observed phenotypes with genome sequences containing multiple antimicrobial- and metal-resistance cassettes.

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GAPDH protein of *Campylobacter jejuni NCTC11168* involves in iron uptake from human ferric- lactoferrin

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Bacterial GAPDH is a highly conserved protein and shares 50% identity with the human homolog. Recently, additional functions beyond the canonical metabolic activity in the glycolysis pathway, of this protein have been alluded to. Studies in other species have reported the contribution of GAPDH to iron acquisition from transferrin and lactoferrin; iron binding proteins. However, there is currently no evidence for *C. jejuni.* This study aimed to determine the role of *C.jejuni* NCTC11168 GAPDH in capturing iron from host ferric-lactoferrin complex. The *gapA* gene is essential in *C. jejuni,* therefore conditional mutants were designed in which the wild type *gapA* allele was knocked out in a constructed *gapA* merodiploid while the second copy is controlled by promoters of differing strength. The wild type*,* merodiploid and mutant *gapA C. jejuni* strains were grown in MEMα medium supplemented with different concentrations of human ferric- lactoferrin. Results show that the strains overexpressing *gapA* displayed increased growth kinetics compared to the wild type *C. jejuni* strain in cultures supplemented with low concentrations of lactoferrin. Additionally, inclusion of anti-GapA antibody in culture supplemented with lactoferrin as the sole iron source blocks completely the growth of all the strains. Similarly, the binding of whole cell *C. jejuni* strains with lactoferrin was reduced markedly when the cells were pre-incubated with anti-GapA. To be concluded, GAPDH of *C. jejuni* can be considered an important agent in iron uptake from lactoferrin and can potentially serve as a specific receptor of lactoferrin.

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Assembly of the Cytochrome *c* Oxidase in *Campylobacter jejuni*

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Campylobacter jejuni, a foodborne microaerophilic pathogen, is the commonest cause of bacterial gastroenteristis in many countries. It uses complex cytochrome-rich respiratory chains, for growth and host colonisation, which includes a pathway to a cbb_3-type cytochrome-*c*-oxidase (CcoNOQP) with a very high oxygen affinity. This oxidase has been shown to be crucial in host colonisation and represents a potential anti-microbial target. The oxidase contains two c-type cytochrome subunits and a bi-nuclear haem-copper active site consisting of Cu(B) and b/b_3-type haems. Insertion of Cu(B) requires an assembly system of copper transporters and chaperones. In *C. jejuni* genes *cj0908 -cj0911* may encode a copper chaperone system. Proteins encoded by *ccoGHIS* play an important role in biogenesis of the cbb_3-type cytochrome-c-oxidase in other bacteria. In *C. jejuni*, we identified Cj1154 as CcoI-homologue, Cj1155 as CcoS-homologue and Cj0369 as potential CcoG-homologue. *Cj1485c and cj1486c* are directly downstream to CcoNOQP, with an unknown function. *cj1483c* may encode a CcoH homologue. We will present data on the roles of these genes in cbb_3-oxidase assembly. Oxidase activity is reduced in most of the mutants and no oxidase activity is measureable in *Δcj1155c* and *Δcj1486c* mutants but activity is restored in *Δcj1155c* on adding excess copper. Cu sensitivity assays showed that the growth defect of the *Δcj1155* mutant is reversed by increasing Cu concentrations. Our data suggest a model for the role of these proteins in the assembly of the oxidase, which will be useful in devising strategies for the inhibition of the oxidase itself or its assembly pathway.

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Structure activity relationships in glycosyl transferases involved in protein O-mannosylation in the Actinobacteria

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Actinobacteria have a protein O-glycosylation system that resembles eukaryotic protein O-mannosylation. Both *M. tuberculosis* and *S. coelicolor* have growth retarded phenotypes when the protein-O-mannosyl transferase (Pmt), a membrane bound enzyme that transfers mannose from a polyprenol phosphate mannose to a target protein, is absent. Moreover, *S. coelicolor* Pmt- mutants are resistant to PhiC31 phage infection and have markedly increased susceptibility to vancomycin and certain beta-lactams. In *S. coelicolor* the phenotype of a strain lacking polyprenol phosphate mannose synthase (Ppm1), is even more susceptible to antibiotics and a Ppm1- mutant in *M. tuberculosis* is lethal. The enzymes Pmt and Ppm1 are therefore possible targets for the isolation of novel antimicrobials against *M. tuberculosis*.

We are characterising *S. coelicolor* Ppm1 as the protein can be expressed and purified in soluble form. Ppm1 normally transfers mannose from GDP-mannose to a polyprenol phosphate acceptor and our aim is to gain an understanding of the structure and function of this enzyme. An analogue mannose acceptor has been chemically synthesised, which will allow kinetic analysis of Ppm1 *In vitro*. Mutant alleles of Ppm1 and Ppm1 homologues from other species have been introduced into a complementation system in a *S. coelicolor* Ppm1- strain, an *In vivo* phenotypic analysis will follow. *S. coelicolor* Pmt is a more challenging target for study as it has 10 predicted membrane spanning helices. We nevertheless, aim to use a similar approach to gain insights to the mechanism of this enzyme in the future.

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Amelioration of methionine protraction through mutagenesis of Corynebacterium sp.

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The present contemplation epitomize to the preferment of corynebacterium strain WA7 for aggrandize methionine production in 250 ml Erlenmeyer flasks .Corynebacterium remote from soil samples collected from sugat factories area in Pakistan . Mutation using ethyle methane sulfonate (EMS 50-300 u/ml) was commence for 10-60 min for mutation . Corynebacterium or its mutant was grown on different fermentation media based on glucose , urea, and molasses to obtain maximum yield of methionine . Acidic ninhydrin method was employed for quantitative analysis of metionine . The amount of methionine produced by wild strain in MF7 fermentation medium by WA7 was 3.0 g/litre , 5.3 g/litre ,6.3 g/litre , and 9.3 g/litre during 24,48,72 and 96 hours incubation under optimum condition respectively in media . which is enhanced by EMS mutant in the same media was 4.5 g/litre , 5.9 g/litre m 7.2 g/litre and 9.5 g/litre by EMSA7 . MF7 was molasses based fermentation medium and consist molasses as key component . For molecular analysis genomic DNA was extracted and 16 S rRNA gene of bacterial isolate was amplified by PCR. The objective of this work is to expound a convictive maximum production of methionine to commercialize this industry in Pakistan .

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Substrate Accelerated Death in Campylobacter jejuni

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Background: Substrate accelerated death (SAD) is a phenomenon that occurs when starved cells are exposed to an excess of the substrate that previously limited their growth. This has been detected in various prokaryotes, including *Klebsiella aerogenes* and *Escherichia coli*. These pathogens were under various types of limitations including but not limited to lactose, glycerol, pyruvate, mannitol and glucose. The phenomenon has also been observed in eukaryotes such as *Saccharomyces cerevisiae.* The aim of this study was to reveal SAD in starved *Campylobacter jejuni* populations.

Methods: *C. jejuni*, a fastidious and microaerophilic pathogen which is considered the most frequent cause of enteric infections, is starved and re-introduced to single carbon substrates it utilizes. The carbon substrates used here include; L-serine and pyruvate.

Results: In this study SAD was observed after a starvation period of 5 hours and in aerobic, anaerobic and microaerophilic conditions. However, the age of the culture or the phase it was in had no effect on the onset of SAD. The phenomenon was also observed in both minimal and complex media, but was not detected in starved *SdaA* (Cj1625c) and *SdaC* (Cj1624c) mutants.

Conclusion: Substrate accelerated death was observed in *C. jejuni* populations and understanding the mechanism of SAD in this pathogen may shed light on this phenomenon in other pathogens, but it may also reveal interesting aspects of *C. jejuni's* ambiguous metabolism

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Horizontally acquired AT-rich genes in *Escherichia coli* cause toxicity by sequestering RNA polymerase

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Horizontal gene transfer permits rapid dissemination of genetic elements between individuals in bacterial populations. Transmitted DNA sequences may encode favourable traits. However, if acquired DNA has an atypical base composition, it can reduce host fitness. Consequently, bacteria have evolved strategies to minimise the harmful effects of foreign genes. Most notably, xenogeneic silencing proteins bind incoming DNA that has a higher AT-content than the host genome. An enduring question has been to understand why such sequences are deleterious. Here, we show that the toxicity of AT-rich DNA in Escherichia coli frequently results from constitutive transcription initiation within the coding regions of genes. Left unchecked, this causes titration of RNA polymerase and a global downshift in host gene expression. Accordingly, a mutation in RNA polymerase that diminishes the impact of AT-rich DNA on host fitness, reduces transcription from constitutive, but not activator-dependent, promoters.

Lamberte *et al.* (in press). Horizontally acquired AT-rich genes in *Escherichia coli* cause toxicity by sequestering RNA polymerase. *Nat. Microbiol.*

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Characterisation of a new *Lactobacillus salivarius* strain with regards to BSH activity

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Bile salt hydrolase (BSH) enzymes are the gateway reaction for bile acid (BA) metabolism by gut microbes (Jones, Begley et al. 2008). BSH removes either glycine or taurine conjugated to a bile acid yielding free BAs, these can then be subsequently further microbially modified. Within the *Lactobacillus salivarius* species alone variation in the BSH proteins have subdivided them into four major groups, all of them are distinctly different (Fang, Li et al. 2009). This study describes a new BSH positive strain of *L.salivarius,* characterized by rRNA and MLST analysis, isolated from porcine faeces. This strain was characterised genetically and it carries three distinct BSH homologues. The collective activity of these enzymes against an array of conjugated bile acids was investigated, by UPLC-MS, to reveal a unique BA signature for this strain. Bile acids are important signalling molecules, within the host they interact with specific receptors influencing many metabolic pathways (Joyce, MacSharry et al. 2014).Our isolate was applied to an *in-vivo* murine model and its potential as a probiotic was assessed. Alterations in bile acid metabolism are associated with numerous disease states therefore full characterisation of strain specific BSH activity, may allow bespoke probiotic matching to ameliorate the effects of such alterations

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E. coli genotype determines impact of folic acid supplementation on *C. elegans*

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Bacterial composition in the gastrointestinal tract (GI) impacts host health. Animals are dependent on their diet and microbiota for the essential micronutrient, folate. Supplementation of synthetic folic acid is recommended to prevent folate deficiency, however there is disagreement in the literature concerning potential health risks, including increased risk of colon cancer. Here we present data suggesting that microbiome variation may explain these discrepancies. We illustrate an indirect route of host folate supplementation via folic acid breakdown into para-amino benzoyl -glutamate (pAB-Glu) that can be taken up by several members of *Proteobacteria* possessing the abgT gene (pAB-Glu Transporter). The further breakdown product, para-amino benzoic acid (pABA), can directly enter bacterial cells through membranes. We show that folic acid preparations contain pAB-GLU and pABA in quantities sufficient to boost *E. coli* folate synthesis in an AbgT-dependent mechanism. Using the *C. elegans: E. coli* model, we show that bacterial uptake of folic acid breakdown products is the main route of host folic acid uptake, and is able to restore animal folate in a *C. elegans* folate deficiency model. We also show that in *E. coli* mutants defective in pABA synthesis, restoration of folate synthesis after folic acid supplementation leads to shortened *C. elegans* lifespan in a mechanism dependent on *E. coli abgT*. This study reveals that depending on bacterial genotype and environmental factors, folic acid supplementation can have both positive and negative consequences for health.

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Metabolic response of *Yersinia intermedia* MASE-LG1 to osmotic stress and ionizing radiation

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Abiotic stresses such as salinity and radiation are some of the major limitations for cell growth. Microorganisms can evolve adaptations to such abiotic stresses. Some of them can be structural, some of these adaptation are metabolic.
Yersinia intermedia MASE-LG1, isolated from an Icelandic lake, can adapt to a wide variety of habitats and rapidly changing environmental conditions. Therefore, we investigated the global metabolic response of *Y. intermedia* to sustained salt stress induced by either $MgSO_4$ or NaCl. In addition to osmotic stress, the effect of ionising radiation was analysed.
After metabolite extraction, the adaptation to salt stress was investigated by systematically identification of already known osmoprotectants. Metabolites were identified which are linked to osmoprotective activity. The investigation of the stress response triggered by the two different salts revealed broad similarities, but the responses are not identical e.g. one salt does not equal another. Beyond the accumulation of osmoprotectants we observed changes of numerous metabolites mainly in the central energy and amino acid metabolism. These adaptations might provide necessary energy and building blocks to fuel processes conveying salt tolerance like the biosynthesis of compatible solutes. When the two stressors (salt and radiation) are applied simultaneously, the response to osmotic stress is favoured and even enhanced. These results point towards a hierarchy in response depending on the type of stress e.g. reaction to the less severe damages can be supressed.
The outcome of this study will have impact on our understanding of **how microorganisms adapt to hostile environmental conditions.**

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Investigating the role of stand alone Rgg transcriptional regulators in *Streptococcus pneumoniae*Author: Bushra Shlla, Peter W. Andrew and Hasan Yesilkaya Department of Infection, Immunity and Inflammation, University of Leicester, United Kingdom

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Rggs are a group of transcriptional regulators found in Gram positive bacteria, and they are important for diverse metabolic functions and virulence. However, their roles in the important human pathogen *Streptococcus pneumoniae* are not known in detail. To assess the functional importance of Rgg homologs in *S. pneumoniae*, targeted mutation was used for deletion of two *rgg* genes, SPD_0999 and SPD_1518, and the mutants were characterised *in vitro* and *in vivo*.

Isogenic Rgg mutants were constructed in *S. pneumoniae* type 2 D39 background using splicing overlap extension method. The growth studies used chemically defined medium supplemented with different sugars under microaerobic condition. The expression of rgg genes was determined using *lacZ* transcriptional assays. *In vivo* significance of Rggs were tested in a mouse model of colonisation, and pneumonia.

In vitro analysis showed that under microaerobic conditions, the mutants were attenuated in growth on mannose and galactose compared to the wild type, whereas their growth profiles were similar to that of wild type CDM containing glucose. Moreover, reporter assays showed that SPD_1518 expression was significantly induced by galactose while SPD_0999 expression was not induced with any of the tested sugars. *In vivo* analysis of Rgg mutants showed that both SPD_0999 and SPD_1518 are required for pneumococcal virulence. All mice infected intranasaly with either Rgg mutant survived significantly longer and had less bacteria in their blood compared to the wild type.

The available results show that Rgg encoded by SPD_0999 or SPD_1518 play a major role in pneumococcal virulence.

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Electrophysiological dynamics during sporulation of *B. subtilis*

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Bacterial sporulation is a well-characterised differentiation process leading to the formation of metabolically quiescent cells. These cells are highly resilient to a series of environmental insults, including antibiotic treatments. A myriad of studies have been conducted on the biochemical and molecular genetic aspects of bacterial sporulation. However, there is limited understanding of the electrophysiological signalling processes on which sporulation relies. Using the spore-forming bacterium B. subtilis as a model organism, we monitored the dynamics of electrical properties of cells at the single-cell level during spore formation. Specifically, we employed fluorescent reporters for intracellular potassium and calcium concentrations, pH, redox state and membrane potential of the mother-cell and prespore compartments. We also systematically analysed the impacts of redox active compounds and ionophores on the successful completion of this differentiation process. Together, our results show that the establishment and maintenance of an electrochemical gradient between the mother cell and prespore is fundamental to the formation of a viable and resistant spore.

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Identification of Staphylococcus aureus cell wall anchored proteins promoting bacterial adherence to cytokeratin 10

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Staphylococcus aureus is a leading cause of skin and soft tissue infection. A risk factor for infection is carriage on the skin. S. aureus can colonise normal healthy skin but the skin of atopic dermatitis patients is colonized to a dramatically higher degree compared with healthy individuals. The first step during skin colonisation and infection is bacterial adhesion to host factors in the outer layer stratum corneum. S.aureus uses surface-located cell wall-anchored (CWA) proteins to adhere to corneocytes at the surface of the stratum corneum. Cytokeratin 10 (K10), a protein expressed by both corneocytes and keratinocytes, has previously been found to be a ligand for the S.aureus CWA protein ClfB. Here, isogenic ClfB-deficient mutants were generated in primary clinical strains of S. aureus taken from the skin of AD patients. ClfB-deficient mutants retained ability to adhere to K10, albeit at slightly reduced levels, suggesting that additional bacterial proteins are facilitating adhesion. In order to identify CWA proteins with affinity for K10, the ligand binding region of a number of S.aureus CWA proteins were expressed as recombinant proteins and tested for binding to K10 by ELISA. One additional K10-binding CWA protein was identified using this approach, FnBPB. An isogenic FnBPB-deficient mutant and a double ClfB/FnBPB-deficient mutant of S.aureus were constructed and their ability to adhere to K10 was tested. Future studies will focus on understanding the interactions between CWA proteins and K10 at the molecular level and determining the contribution of K10 binding to bacterial adhesion to the stratum corneum.

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The rate-determining step of arsenite oxidase is the reduction of its electron acceptors.

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Arsenic is a widely distributed environmental toxin that currently poses a threat to approximately 150 million people worldwide. The respiratory enzyme arsenite [As(III)] oxidase (Aio) from *Rhizobium* sp. str. NT-26 is currently being developed as a biosensor for arsenite detection in drinking water. Aio consists of two subunits: the large subunit contains a molybdopterin guanine dinucleotide and a [3Fe-4S] cluster; the small subunit contains a Rieske [2Fe-2S] cluster. Various chemicals (e.g. dichlorophenolindophenol) and proteins (e.g. cytochrome *c*) can serve as electron acceptors to Aio. Stopped flow spectroscopy was used to determine that the reduction of the electron acceptors was rate-limiting.

The small subunit mutant, AioB-F108A, was found to specifically reduce activity with cytochrome *c* (slightly increasing the activity with DCPIP). Isothermal titration calorimetry and steady-state kinetics were used to investigate the interaction of WT and F108A arsenite oxidase with cytochrome *c*. It was found that F108A had similar affinity to WT for cytochrome *c* meaning that residue F108 plays a critical role in determining the rate of electron transfer between the two proteins.

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A unified hypothesis for indole signalling in bacteria

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Indole is an aromatic molecule with diverse signalling roles that is secreted by over 85 species of bacteria, including Escherichia coli. The effects of indole signalling are concentration dependent, ranging from altered patterns of gene expression (<1mM) to the induction of cellular dormancy (5mM). We propose a unified model of indole signalling based solely upon its action as a proton ionophore, where in the range 0-2 mM, indole-induced proton flux decreases intracellular pH, while 3-5 mM reduces membrane potential. We argue that the various phenotypic effects of indole described in the literature are secondary to these changes. Previous work in our laboratory using the anionic voltage-indicating dye, Oxonol VI, has shown that indole at 3 mM and above reduces the electrical potential across the E. coli cytoplasmic membrane. This observation has been confirmed using the cationic dye 3, 3`-Diethyloxacarbocyanine iodide, DiOC2(3). Additionally, we used the cytoplasmic pH indicator “pHlourin” to compare the effect of indole on the intracellular pH of E. coli in a wild-type strain and a tryptophanase deletion mutant that has lost the ability to produce indole. The regulation of cytoplasmic pH was significantly affected by the production of indole. The effect was seen both in stationary-phase and exponential-phase cultures, even though the latter contained a very low concentration of indole (< 20 µM). Our work identifies the cytoplasmic membrane as the primary target of indole and identifies a mechanism of indole action equally applicable to indole-producing and non-producing bacteria.

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Rgg transcriptional regulators have a role in pneumococcal survival and virulence

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Streptococcus pneumoniae causes a range of life-threatening diseases in different human tissues, which suggests that the microbe has effective mechanisms to sense and respond to environmental stimuli. However, the regulatory mechanisms required for pneumococcal adaptation are poorly understood. The Rgg family of proteins are transcriptional regulators that have been shown to be important for survival and virulence in other streptococci but their role in the pneumococcus is unknown. The pneumococcal type 2 D39 strain has 5 different Rggs, and two of them, SPD_0144 (Rgg144) and SPD_0939 (Rgg939), are associated with genes coding for a short hydrophobic peptide (shp). It has been suggested that Rgg-SHP circuits are components of quorum sensing systems in Gram positive bacteria. Therefore, the objective of this study is to determine Rggs' role in the pneumococcal biology and investigate if Rggs are part of new quorum sensing systems in *S. pneumoniae*.

Site directed deletion of putative rgg genes coded by SPD_0144 and SPD_0939 was done by overlap extension PCR. Routinely, chemically defined medium (CDM) was used to characterise the mutants. Rgg deficient mutants were tested for their resistance to paraquat, their ability to utilize different sugars, and virulence in an experimental murine infection model. Moreover, Rgg deficient mutants were compromised in their ability to use non-glucose carbohydrates, exhibited susceptibility to oxidative stress. In addition, both Rgg144 and Rgg939 are involved in pneumococcal virulence. The expression of shp0144 and shp0939 is induced by truncated SHP peptides, and Rggs induce expression of shp genes.

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Metabolic regulation of the Type Three Secretion System (T3SS) in *Pseudomonas aeruginosa*

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Type three secretion system (T3SS) has a well-established role in *Pseudomonas aeruginosa (P. aeruginosa)* infections. The pathogen uses the T3SS to inject toxins directly into the recipient's cytoplasm, thereby subverting host cell function leading to tissue damage and death. In other microorganisms, pyruvate kinase activity has been linked with the control of pathogenicity. In *P. aeruginosa*, pyruvate kinase is encoded by two isozymes, PykA and PykF. The main objective of this work was to characterize these isozymes and to understand their role in regulation of T3S. Kinetic parameters of the purified proteins revealed that PykA and PykF have relatively similar K_{half} values for the substrate, phospho*enol*pyruvate (1.08 mM and 0.77 mM, respectively), whereas the K_M values for ADP differed by ca. two-fold (0.05 mM and 0.10 mM, respectively). Both isoenzymes require Mg^{2+} for optimal activity, although unlike pyruvate kinase from other bacterial species, they do not require K^+. No enzyme activity was detected in a *pykA* mutant or in a *pykA-pykF* double mutant, whereas a *pykF* mutant was similar to the wild-type for pyruvate kinase activity. Consistent with this, transcriptional activity assays confirmed that the promoter driving *pykA* expression is much more active than the promoter driving *pykF* expression. However, although a *pykA-pykF* double mutant failed to express components of the T3SS compared with the wild type or *pykA* or *pykF* single mutants. In conclusion, although PykA is apparently the dominant isozyme in pyruvate metabolism, it seems that PykA and PykF play a combined role in regulation of the T3SS.

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DarTG is a novel toxin-antitoxin system that catalyses the reversible ADP-ribosylation of DNA

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Many prokaryotes use a variety of toxin-antitoxin (TA) systems to regulate cellular processes related to the general stress response, such as defense against phages, growth control, biofilm formation, persistence and programmed cell death. Here we describe a novel TA system present in a variety of bacterial species including the global pathogen *Mycobacterium tuberculosis*. Whereas the toxin of the system (DarT) was originally classified as a domain of unknown function, the antitoxin (DarG) had been correctly identified as a putative macrodomain protein. We present the solved X-ray crystal structures of the macrodomains of *Thermus aquaticus* and *M. tuberculosis* DarG, and demonstrate that DarT specifically modifies thymidines on single-stranded DNA in a sequence-specific manner by a nucleotide type modification called ADP-ribosylation. We also report that DarG can remove this modification and is an essential enzyme needed for the survival of the bacteria that encode this TA-system. Our results show that DARTG is the first example of an enzyme pair that catalyses the reversible ADP-ribosylation of DNA and we anticipate potential therapeutic benefits by targeting this novel enzyme-enzyme TA system in bacterial pathogens such as *M. tuberculosis*.

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A possible reclassification of *Aquificaceae* and proposal for creation of a new family *Hydrogenivirgaceae* fem. nov. containing single genus *Hydrogenivirga* within the order Aquificales – A Bioinformatics approach

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The family *Aquificaceae* belongs to the order Aquificales of the class Aquificae under the phylum Aquificac. The family is constituted with hyperthermophilic, chemolithotropic or microaerophilic or aerobic eubacterial genera. They represented the deepest rooting among eubacteria. Their Genomes are characterized by having rather low GC-content, ranging between 35%-51.3%. In order to find out a common molecular signature of these bacteria, the codon usage profiles, COA and CAI values of them were calculated. Furthermore, a few physical parameters of the bacteria and their genomes, such as the number of genes present, average gene length, highest length of gene (bp), lowest length of gene (bp), habitat/isolation, optimum growth temperature (˚C), genome size (Mb) were also taken into account. An overall uniformity was found in all the genomes except *Hydrogenivirga sp* which has a larger genome and possesses mesophilic growth habit. These results suggest that mesophilic adaptation require more genes than thermophilic adaptation. The thermophilic organisms of the early earth accumulate genes to adopt in mesophilic condition. Furthermore, genus *Hydrogenivirga* was suggested to be reclassified and possibly to be moved to a new family *Hydrogenivirgaceae* fem. nov. due to its uniqueness in genome property.

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Characterisation of the OrbS sigma factor from Burkholderia cenocepacia

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The cystic fibrosis pathogen *Burkholderia cenocepacia*, produces the siderophore ornibactin to capture iron from the environment. This siderophore has high affinity for ferric iron and contributes to pathology in animal models of respiratory infection and to virulence. The Ferrirc uptake repressor (Fur)-regulated OrbS Extracytoplasmic (ECF) sigma factor is required for transcription of the ornibactin gene cluster from three promoters. Unlike most ECF sigma factors, OrbS does not have a candidate anti-sigma factor to sequester its activity when iron is plentiful. Inactivation has only been demonstrated at the transcriptional level with Fur binding to the *orbS* promoter. However, the activity of the OrbS dependent promoters in high iron are shut down even in strains lacking the Fur repressor, suggesting an additional regulatory mechanism.
Here, we provide evidence that 4 cysteine residues within the C-terminal region of OrbS are important for OrbS regulation in both *in vitro* and *in vivo* assays. At high iron, a *B. cenocepacia* strain containing the *orbS CtetraA* allele was less susceptible to ornibactin synthesis repression than the wild type strain. This phenotype was further exacerbated in an *orbS CtetraA Δfur* mutant.
We conclude that OrbS is a novel direct intracellular iron sensor with a C-terminus that assumes the role of an on-board anti-sigma factor under iron-replete conditions.

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The Fitness Cost of Triclosan Resistance

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Triclosan is a broad spectrum antimicrobial used in a wide variety of products including consumer goods, cosmetics and medical devices. The antimicrobial activity of triclosan is attributed to the inhibition of FabI, a type II fatty acid synthesis enzyme. Reduced susceptibility towards triclosan has been observed in *Escherichia coli* due to point mutations in the enzyme[1].

Enzyme kinetics elucidated that the mutants are not as efficient in reducing the double bond within the enoyl moiety during chain elongation[1]. We hypothesised this would have a significant fitness cost as the ability of the cell to adapt and maintain the membrane could be impaired. Our results suggest that the previously published mutants have an impaired growth, with stationary phase being extended. In addition, a decline in OD_{650nm} readings was observed in all mutants except F203L after 18 hours, suggestive of cell death, as this was not seen in *E. coli* containing the wild-type enzyme. All mutants when in direct competition with wild type, other than F203L, do not grow and in fact after a 6 hour period begin to undergo cell death. Furthermore, the introduction of triclosan did not lead to a re-emergence of the mutant phenotype after direct competition.

Our results suggest that the mutations within FabI attributed with triclosan resistance have a significant fitness cost, and the mutants do not remain as “persister cells” when the antimicrobial pressure is removed.

References

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Role of hexonate metabolism in resistance to egg white in *Salmonella enterica* serotype Enteritidis

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Salmonella Enteritidis (*S*E) is an important serovar causing disease in many animal species as well as humans. Poultry and poultry products are the major sources of human infection with S*E*. Identifying *S*E genes that confer resistance to egg white might help to explain the specific ability of *S*E to survive under the harsh conditions provided by egg white. Our previous work revealed global expression changes of *S*E in egg-white medium. Surprisingly, three distinct gene clusters, likely representing a novel regulon, were strongly induced: the *dgoRKADT* operon, the *uxuAB-uxaC* operon and the SEN1433-6 genes. These genes are related to utilisation of hexonates and hexuronates, and have not been previously reported to possess any role in egg-white survival. The aim of this work is to determine the role of these genes in the survival of *S*E. Nine of the putative promoter regions of interest were tested for expression by generation of *lacZ* fusions. Activities were variable, with highest observed for SEN1436 (encoding for putative dehydratase) and SEN2977. The SEN1436-*lacZ* fusion was selected for subsequent work for the study of environmental regulation. Upon exposure to egg white (EW), SEN1436 expression was induced up to 60 fold. However, egg-white filtrate (lacking proteins >10 kDa) had no marked effect on expression. This result suggested that EW proteins are responsible for the induction of the hexonate genes during EW exposure. Experiments involving exposure to isolated EW proteins (lysozyme, ovalbumin, conalbumin and ovomucoid) showed that that major EW protein affecting SEN1436 expression is lysozyme (14 fold).

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Purification and characterization of RhlR; a transcriptional regulator involved in quorum sensing in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a multi-drug resistant, opportunistic human pathogen. The organism readily forms biofilms and produces an arsenal of potent secreted virulence factors, which facilitate infection of the host. The expression of these virulence factors are largely mediated by a cell-cell communication mechanism called quorum sensing (QS). Over the last two decades, much has been learned about the molecular mechanism(s) of QS in *P. aeruginosa*, and it is now clear that three key signalling sub-systems are involved; the las, PQS, and rhl pathways. Although we now know much about the las and PQS pathways, the rhl pathway has stubbornly refused to submit to biochemical investigation. This is significant because it is now becoming clear that rhl signalling plays a key role in virulence regulation, as well as enabling survival of the pathogen in oxygen-limited environments. The transcriptional regulators involved in las and PQS signalling (LasR and PqsR, respectively) are relatively well-characterized. However, very little is known about the main rhl sub-system receptor, RhlR. This is primarily because RhlR is exceptionally difficult to express; the gene encodes a large number of "rare codons", leading to sub-optimal translation. However, we have found that codon optimization overcomes this problem, allowing large amounts of RhlR to be expressed in the soluble fraction of cells. This finding, opening the way towards detailed structural and biochemical characterization of the protein, is important because we have recently obtained evidence that suggests RhlR not only binds an *N*-acyl homoserine lactone (BHL) ligand, but also the alkyl quinolone, PQS.

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***Bacillus licheniformis* contains three PerR paralogues involved in hydrogen peroxide sensing response**

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The ferric uptake regulator (Fur) family proteins include sensors of Fe (Fur), Zn (Zur), Mn (Mur), Ni (Nur), heme (Irr) and peroxide (PerR). The Gram positive bacteria such as *Bacillus subtilis*, *Listeria monocytogenes* and *Staphylococcus aureus* encode three Fur family proteins: Fur, Zur, and PerR. In this study, we identified five Fur family proteins from *B. licheniformis*: two novel PerR-like proteins (BL00690 and BL00950) in addition to Fur (BL05249), Zur (BL03703), and PerR (BL00075) homologues. All of the five *B. licheniformis* Fur homologues contain a structural Zn^{2+} site composed of four cysteine residues like many other Fur family proteins. Importantly, mass spectrometry studies indicate that the histidine residues of PerR-like proteins (BL00690 and BL00950) as well as $PerR_{BL}$ (BL00075), but not Fur_{BL} (BL05249) and Zur_{BL} (BL03703), can be oxidized by H_2O_2 with different sensitivity. These data suggest that the three PerR subfamily proteins can sense H_2O_2 by histidine oxidation. We also show that PerR2 (BL00690) has a PerR-like repressor activity for PerR-regulated genes *in vivo*, indicating the presence of dual regulation mode of PerR-regulated genes by two PerR homologues, $PerR_{BL}$ and PerR2. Despite the lack of canonical repressor function for PerR3 (BL00950), our data provide evidence that the role of PerR3 is likely to be linked with $PerR_{BL}$-mediated H_2O_2 sensing mechanism.

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Biochemical Analysis of SmoS from *Sinorhizobium meliloti*

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Sugar alcohols, also called polyols, are carbohydrates that are formed from the reduction of an aldose or ketose sugar. The most common polyols found in vascular plants are sorbitol, mannitol and galactitol (dulcitol). Plant derived carbon compounds are secreted from the root tissue into the soil creating an environmental niche called the rhizosphere. The ability of rhizosphere dwelling symbiotic bacteria, such as *Sinorhizobium meliloti*, to compete for nodule occupancy can be affected by their ability to catabolize sugar alcohols.

Analysis of the *S. meliloti* genome revealed the presence of the *smo* locus, a region predicted to be involved in the catabolism of several sugar alcohols. In this work, the putative sorbitol dehydrogenase SmoS is biochemically characterized using classical Michaelis-Menten kinetics as well as X-ray crystallography techniques. SmoS is a member of the short chain dehydrogenase/reductase (SDR) protein family. It is a 257 amino acid peptide with a molecular weight of 27 kDa that exhibits NAD^+ dependent dehydrogenase activity on sorbitol and galactitol. The catalytic tetrad of Asn-Ser-Tyr-Lys, which is conserved in most SDR family proteins, is present.

The biochemical analysis will provide insight into physiological processes such as the contributions of sugar alcohols to free living bacteria and symbiosis. Additionally, it better defines the parameters of the *S. meliloti* carbon catabolism model as well as the *Sinorhizobium-Medicago* symbiotic model.

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Structural basis for ligand recognition and specificity by sensory domain of Campylobacter jejuni transducer-like protein Tlp3

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In Campylobacter jejuni, chemotaxis and motility have been identified as important virulence factors that are required for host colonisation and invasion. Chemotaxis involves recognition of external chemical cues by the sensory domain (SD) of chemoreceptors. Recently, we determined the crystal structure of Tlp3-SD in complex with the attractant isoleucine, which is directly recognised through one of the two putative binding pockets present in this SD. In this work, we performed a high-throughput screening of potential ligands to identify novel signal molecules detected by Tlp3 and the crystal structure of the receptor SD in complex with the ligands were also analysed.
In total, 248 different compounds were screened. From these, we identified five molecules that potentially interact with Tlp3-SD: L-leucine, L-valine, L-norvaline, alanine-threonine peptide, methyl pyruvate and 2'-deoxycytidine. To date, we have confirmed that L-leucine, L-valine and L-norvaline bind to Tlp3-SD with a KD of 191, 409 and 203 µM, respectively. These amino acids have a similar structure which resembles that of isoleucine (KD= 86 µM), and as expected, are also recognised in a similar fashion. The Tlp3-SD structure comprises membrane-distal and membrane-proximal Per-Arnt-Sim (PAS)-like domains, each of which contains a putative binding site. Analysis of the Tlp3-SD complex structures showed that the three molecules are bound to the membrane-distal PAS-like domain with extensive interactions formed between the protein and both side-chain and main-chain of the amino acid ligands. This structural analysis provides a molecular basis for understanding the mechanism of ligand recognition and specificity of the Tlp3 receptor.

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Characterisation of transporters that allow soil bacteria to scavenge natural and synthetic phosphonates from the environment

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Phosphonates are compounds characterised by a direct C-P bond and are environmentally abundant, thought to contain around 4% of the total phosphorus in some soils. This class of compounds contains abundant naturally occurring chemicals like 2-aminoethylphosphonate (2-AEP) and the important synthetic herbicide glyphosate. Bacteria have evolved systems to scavenge the phosphorus from these compounds to use as a nutrient. Transport of 2-AEP in most Gram negative bacteria is mediated by an ABC transporter encoded by the phnCDE genes. Herein we seek to discover additional phosphonate transporters present in soil bacteria, particularly ones that have been shown to uptake and catabolise glyphosate in the environment. A series of strains of the rhizosphere bacterium Sinorhizobium meliloti were tested for their ability to use different phosphonates as the sole P source. Mutation of the phnC gene limits the range of phosphonates that S. meliloti can grow on, but does not abolish growth on phosphonates altogether, suggesting the presence of additional transporters for 2-AEP. In parallel, a biochemical approach studying the substrate binding protein (SBP) component of ABC transporters revealed a number of proteins that can bind natural phosphonate with low μM affinity. Significantly, some of these appear to also recognise glyphosate and are being considered as potential scaffold for a glyphosate biosensor.

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THE ROLES OF THE THREE IRON-STORAGE PROTEINS OF *ESCHERICHIA COLI* IN SUPPORTING LOW-IRON GROWTH AND REDOX STRESS RESISTANCE

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Organisms must balance their iron requirement to achieve iron homeostasis using various mechanisms including sequestration of excess cellular iron by iron-storage proteins. Such proteins can also act to counter redox stress imposed by excess cellular iron. In *Escherichia coli*, there are three iron-storage proteins: ferritin A (FtnA), bacterioferritin (Bfr) and the 'DNA-protection during starvation' (Dps). Experiments were conducted to further investigate their respective functions and to determine whether these proteins are mutually interchangeable. This aim was progressed by generating a triple mutant of iron-storage genes (BW25113 Δ*ftnA* Δ*dps* Δ*bfr*) by P1 transduction confirmed by PCR and Western blot; this mutant was then complemented separately with iron-storage proteins each under the control of a rhamnose-inducible promoter. The results showed that Dps and Bfr pre-induction enhances growth (6.5- and 8-fold, respectively) of the triple mutant in the presence of the H_2O_2, indicating that both these proteins provide redox-stress resistance. However, FtnA pre-induction does not appear to provide resistance to redox stress. Pre-induction of Bfr increased growth (~2.5-fold) under iron restriction with DTPA; however, neither Dps nor FtnA pre-induction caused any notable growth advantage under Fe-restriction. Thus, only Bfr acted to provide an apparent iron source that could promote iron-restricted growth. Subsequent studies indicated that the weak FtnA expression is due to rapid turnover of the protein through 'N-end rule dependent' degradation. Indeed, replacement of the second FtnA amino acid (LàA/K) resulted in a ~10-fold increase in FtnA levels. Thus, FtnA iron release may be partly dictated by ClpS-AP dependent degradation.

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It's all Rel-ative. Comparative analysis of bacterial (pp)pGpp alarmone synthesizing proteins

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Background
During the 'stringent response' bacteria produce phosphorylated guanine nucleotides known as 'alarmones' (pppGpp, ppGpp and pGpp). These alarmones modulate bacterial cell physiology to promote survival. However, the precise activities of the protein families implicated in the synthesis and/or degradation of (pp)pGpp alarmones remain to be fully-elucidated.
Objectives
To compare and contrast the biochemical properties of RelA/SpoT Homologue (RSH) and small alarmone synthase (SAS) proteins from diverse bacterial species of relevance to human health.
Methods
Selected SAS and RSH protein homologues encoded by Staphylococcus aureus, Enterococcus faecalis, Fusobacterium nucleatum, Actinomyces gerencseriae and several other bacterial species were cloned, expressed, purified and biophysically-characterized using standard procedures. Their respective abilities to synthesize and degrade alarmones were systematically characterized. Enzymatic kinetic parameters were established by quantifying reaction products using liquid chromatography.
Results
The tetrameric SAS homologues preferentially utilized GDP over GTP or GMP as the acceptor for alarmone synthesis, with ATP solely-utilized as diphosphate donor. SAS proteins also accepted inosine nucleotides (ITP, IDP, IMP) as acceptors to differing extents. The alarmone-synthesizing activities of some SAS proteins were not notably stimulated by pppGpp or ppGpp. Regarding the RSH proteins, all RSH homologues tested rapidly hydrolyzed pppGpp, ppGpp and pGpp; with activities essentially-dependent on Mn(II) ions. Notably, certain RSH proteins could utilize GMP as substrate for the production of pGpp.
Conclusions
Certain species of bacteria may employ both RSH and SAS proteins for the synthesis of pppGpp, ppGpp or pGpp. Furthermore, RSH proteins have the capability to efficiently hydrolyze any (pp)pGpp formed within the cell.

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Determining the mechanics of Staphylococcus aureus comet formation

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It has been shown that Staphylococcus aureus can move across surfaces via two behaviours: 1) passively moving across the surface of agar plates in a process called spreading (a form of sliding motility), and 2) the formation of dendrites by 'comets' of slime-covered aggregates of cells that move in a manner that share many similarities with a form of active motility known as gliding (especially with certain bacteria that also engage in what are also termed 'comets'). Both behaviours are dependent on surfactant (the Phenol Soluble Modulins, which are regulated by the agr quorum sensing system).
As both spreading and comet formation occur under similar conditions and have similar nutritional requirements we set out to determine the experimental variables that determine the formation of comets. We found that spreading occurred over a wide range of agar concentrations, drying times and agar types (amongst other variables) but comet formation occurred over a much narrower range of conditions and only on specific agar types. We also made further observations of the comets themselves: we found that the staphylococci within the comets arranged themselves differently to the staphylococci in the rest of the colony and that the comets were capable of moving upwards against gravity for extended distances. This work contributed towards generating better defined and more reproducible comet and spreading assays as well as revealing further insights into comet behaviour.

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