OFFERED TALKS

Screening for Novel Inhibitors of *Tannerella forsythia* and *Porphyromonas gingivalis* Sialidases

Raphael Galleh, Daniel Lambert, Graham Stafford

The University of Sheffield, Sheffield, United Kingdom

Abstract

- Periodontitis is a chronic inflammatory disease characterized by the weakening of the supporting tissues surrounding the teeth and subsequent tooth loss. *Porphyromonas gingivalis* and *Tannerella forsythia* are associated with periodontitis and are shown to secret sialidases. Sialidases are glycosyl hydrolase (GH33) enzymes, which catalyze the hydrolysis of terminal sialic acid (desialylation) from extracellular glycoconjugates. The NanH and SiaPg of *T. forsythia* and *P. gingivalis*, contribute to bacterial pathogenesis by promoting the formation of biofilms, invasion of epithelial cells, immune modulation, and colonization. Screening for novel inhibitors of sialidases could serve as a novel therapeutic approach. NanH and SiaPG were purified using HisTag low-affinity chromatography, and the sialidase activity was tested using MUNANA, as substrate. Docking of the respective inhibitors into the active-site pockets of NanH-apo [PDB ID 7QYP], and SiaPg [PDB ID 8GN6] was conducted using AutoDock Vina and visualized in PyMol. Inhibition studies show Epicatechin gallate, Palmatine, and Berberine chloride, and transition-state analogues including Oseltamivir, Siastatin B, and DANA as well as the mechanism-based di-fluoro sialic acid analogues, 2e3aDFNeu5Ac and 2e3aDFNeu5Ac9N3 to have significant inhibitory properties. Also, molecular docking enables clear identification of the key residues that are required for substrate binding and catalysis, including the arginine triad (Arg423/194, Arg487/460, Arg212/398), the conserved nucleophilic dyad Tyr518/488 and Glu407/382, and the acid/base residue Asp280/219 for NanH and SiaPg, respectively. These findings provide the structural basis for further design of effective inhibitors to target NanH and SiaPG to fight against *T. forsythia* and *P. gingivalis*-associated oral diseases such as periodontitis.

Multimodal optical mesoscopy for the study of biofilm infection in palatine tonsils

Megan Clapperton[1], Catalina Florea[2], Tash Kunanandam[2], Catriona Douglas[3], Gail McConnell[4]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]Royal Hospital for Children, Glasgow, United Kingdom. [3]Queen Elizabeth University Hospital, Glasgow, United Kingdom. [4]Strathclyde Institute for Pharmacy and Biomedical Sciences, Glasgow, United Kingdom

Megan Clapperton

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

Biofilms are a leading cause of chronic infections and are known to be more resistant to antibiotic treatment than planktonic bacteria. Bacterial biofilms have been studied in tonsil-related diseases, but little is known about their spatial location and size distribution. We present optical mesoscopy with the Mesolens as a tool to study the location and size of bacterial biofilms in whole mounts of live pediatric palatine tonsils up to 5 mm x 5 mm x 3.0 mm in size, with subcellular resolution of 0.7 μm laterally and 7 μm laterally. We have developed reflection confocal mesoscopy to reveal topography of the unstained tissue and we have simultaneously applied confocal fluorescence mesoscopy to identify the presence of both planktonic bacteria and biofilms within a single acquisition.

Whole mounts of live tonsil tissue were stained with Vancomycin-BODIPY FL conjugate to visualise gram-positive bacteria and biofilms relative to the tonsil host, then imaged with the Mesolens. We quantify the bioburden using a simple image processing pipeline. Biofilms were found to be equally spread across tonsil surface, crypts and core, regardless of patient condition. The benefit of mesoscale imaging is demonstrated through the ability to visualise biofilms within a much larger tissue volume than can be studied at equivalent spatial resolution with a conventional confocal microscope. This innovative imaging method to assess biofilm burden in live tissue could be translated to other fields of disease where biofilm infection is clinically relevant.

Investigating the fractal nature of channels within *E. coli* biofilms with altered cell phenotypes

Beatrice Bottura, Liam Rooney, Paul Hoskisson, Gail McConnell

Unviersity of Strathclyde, Glasgow, United Kingdom

Beatrice Bottura

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

Nutrient-transporting channels are found throughout mature *Escherichia coli* biofilms, where they form intricate fractal patterns. We previously found that the width and distribution of these channels is impacted by environmental growth conditions, but we never explicitly quantified their fractal morphology. Because intra-colony channels are an emergent property of *E. coli* biofilm formation, we hypothesised that their morphology would also be affected by shape changes in the constituent cells.

We tested this hypothesis by imaging mature biofilms formed by six fluorescent *E. coli* strains with different morphological phenotypes (normal, long, chained and wide) using confocal microscopy. We quantified the internal biofilm architecture using the FIJI plugin ComsystanJ to measure the box-counting fractal dimension of channel networks. We also investigated the lacunarity of the biofilms, which describes empty spaces in a pattern.

Lacunarity did not vary significantly between biofilms formed by different morphotypes, likely due to limited channel width variations across the strains. On rich media, the fractal organisation of channels inside the biofilm was linked to cell phenotype. The fractal geometry of biofilms grown on rich media was comparable to that of nutrient-deprived biofilms, except for the Δ*ompR* strain, where box-counting dimension was significantly lower on minimal medium ($p < 0.0005$). This difference could be due to the role of OmpR in osmotic stress regulation and outer membrane permeability, which affects nutrient metabolism.

Our findings suggest that fractal geometry is a valuable tool to quantify biofilm internal patterns arising in response of environmental and morphological changes to constituent cells.

Sensing Across Scales: Quantifying the Oxygen Microenvironment in Biofilm Transport Channels to Inform Better Therapeutics

Liam Rooney[1], Beatrice Bottura[1], Lindsey Florek[2], Lars Dietrich[2], Gail McConnell[1]

[1]University of Strathclyde, Glasgow, United Kingdom. [2]Columbia University, New York City, USA

Liam Rooney

Presenter pronouns (eg. she/her, he/him, they/them)
He/Him

Abstract

Understanding the chemical microenvironment in biological systems informs the development of therapeutic applications. Biofilms are dense, complex microbial communities; they are drivers of antimicrobial resistance, contribute over $4 trillion in global economic impact, and are associated with over 80% of chronic infections. New treatments are urgently required. We discovered an intricate network of channel structures which transport nutrients around the biofilm using the Mesolens; a giant microscope capable of imaging multi-millimetre-sized biofilms while resolving every single cell. We hypothesised that the transport function could be exploited for targeted antibiotic delivery but first, we must understand the channel microenvironment to ensure antibiotic molecules are not destroyed by oxidation.

We developed a dual-pronged approach to measure the local oxygen concentration in intact mature biofilms. We quantified the oxygen concentration inside channels and in the rest of the biofilm using the Unisense Microsensor platform. In parallel, we delivered fluorescent oxygen-sensing nanoparticles directly into biofilm channels, where they were imaged using confocal laser scanning microscopy to provide a ratiometric measure of oxygen contraction along the channels. Using both methods found that the oxygen concentration in channels did not significantly differ from the rest of the biofilm, but that it decreased proportionally along the length of the channels towards the core. These findings indicate that no chemical protection is required to tailor antibiotics for targeted channel delivery, and that we can repurpose existing antimicrobials to develop new and impactful delivery methods and therapeutics.

Deducing clonal complex population structure from gene content

Emily Fotopoulou

UKHSA, London, United Kingdom. University of Warwick, Coventry, United Kingdom. HPRU- GI, Liverpool, United Kingdom

Abstract

The UK Health Security Agency holds the UK's Listeria monocytogenes surveillance database containing whole genome sequencing (WGS) data and associated meta-data from clinical cases, food and food production environments. WGS has proven invaluable in identifying key genes, facilitating L. monocytogenes pathogenic success. This work tests for a link between key genes and clone types, through Multiple Correspondance Analysis (MCA) statistical model.

Virulence factors were tested to explore any covariances between lineages and clonal complexes (CC). MCA was performed using the GeneFinder mapping program outcomes, as categorical variables. CC and lineages were supplied as additional variables, but not used for the analysis. The dataset contained isolates from lineage I; CC1(n=237), along lineage II; CC8 (n= 209) and CC9(n=362).

MCA recovered the clonal structure of isolates, with clear groupings in isolates, corresponding to the known CC type. We also recovered the association between isolate clusters, and gene clusters defining each CC. Finally, we identified other isolates and genes without clear population structure, which seemed to correspond to genetic outliers.

Further investigation is needed to determine whether those isolates actually correspond to different phenotypes. This study highlights the power of MCA as a de-novo method to characterise bacteria populations from their gene content.

Detection and characterisation of Salmonella enterica serovar Infantis (eBG31) harbouring blaCTX-M-1 causing clinical disease in humans in England.

Matthew Bird[1,2], David Grieg[1,3,4], Satheesh Nair[1], Claire Jenkins[1,5], Gauri Godbole[1], Anaïs Painset[1], Vivienne DoNascimento[1], Saheer Gharbia[1,6], Marie Chattaway[1,6]

[1]National Infection Service, London, United Kingdom. [2]NIHR Health Protection Research Unit (HPRU) in Healthcare Associated Infections and Antimicrobial Resistance, Oxford, United Kingdom. [3]Division of Infection and Immunity, The Roslin Institute and Royal (Dick) School of Veterinary Studies, Edinburgh, United Kingdom. [4]Health Protection Research Unit in Genomics and Enabling Data, Warwick, United Kingdom. [5]Health Protection Research Unit in Gastrointestinal infection, Liverpool, United Kingdom. [6]Health Protection Research Unit in Genomics and Enabling Data, Liverpool, United Kingdom

Matthew Bird

Presenter pronouns (eg. she/her, he/him, they/them)
he/him

Abstract

There is an increasing number of reports on the emergence of Salmonella enterica subspecies enterica serovar Infantis, eBG31, harbouring plasmids carrying multiple resistance determinants, termed 'plasmid of emerging S. Infantis' (pESI). Investigating the UK Health Security Agency (UKHSA) archives identified 2169 S. Infantis eBG31 isolates between 2014 and 2021. Of those 2169 isolates 78 encoded any blaCTX-M variant – blaCTX-M-65 (59 isolates), blaCTX-M-1 (18 isolates) and blaCTX-M-14 (1 isolate). The 18 cases of eBG31 S. Infantis encoding blaCTX-M-1 nine cases were female, six cases were male and three were unknown. Only two cases reported recent travel with only one of these two reporting a travel destination, which was Italy.

Single nucleotide polymorphism typing (SNP) typing was conducted identifying that 7 of the 18 blaCTX-M-1 encoding isolates fell within the same 5 SNP single linked cluster, indicating close genetic similarity and likely from a common source, although no common source could be identified. We retrospectively re-sequenced 12 of these 18 isolates using Oxford Nanopore Technologies to characterise the mobile genetic elements on which key AMR determinants are located. All resistant determinants were located within one of two resistant sites on the pESI-like plasmid which had been previously outlined by Tate et al. (2017). However, unlike previous studies who identified blaCTX-M variants in site 1 we identified blaCTX-M-1 in site 2. Understanding these mobile elements allow us to monitor the emergence and spread of important AMR determinants (such as blaCTX-M variants) in all Salmonella species at both the local and global level.

Virus-mediated immunotherapy targeting Severe Acute Respiratory Syndrome Coronavirus 2

Samantha Garcia Cardenas, Gemma Swinscoe, Russell Hughes, Adel Samson, Stephen Griffin

University of Leeds, Leeds, United Kingdom

Samantha Garcia Cardenas

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

The COVID19 pandemic caused by SARS-CoV2 is propagated by unmitigated prevalence and evolution of the virus to escape from adaptive immunity. Nevertheless, subversion of innate immune responses plays an important role in both the establishment of infection as well as progression to severe COVID. Notably, delayed and downregulated responses to type I interferons (IFN) are integral to successful virus transmission.

Oncolytic viruses are an important mode of immunotherapy against cancer. However, we previously showed that type 3 Dearing strain oncolytic reovirus ("reo"), was able to target both malignant cells as well as underlying oncogenic virus infections, such as hepatitis C and B viruses, and Epstein Barr virus. Critically, this was due to rewiring existing innate immune evasive patterns and ensuing induction of a potent type I IFN response.

Reo has never reached a dose-limiting toxicity in cancer patients, displays pulmonary tropism, and is administered deep into the lungs via a nebuliser. Thus, we hypothesised that Reo may be a safe, effective way to deliver antiviral immunotherapy to the infected airway, avoiding the potential toxic effects of systemic/inhaled IFN.

Live and uv-inactivated Reo particles elicit contrasting antiviral responses in cell lines derived from alveolar and bronchial epithelia. However, tailoring agents leads to efficient induction of type I IFN and ISGs. We describe comparative therapeutic efficacy and characterisation of innate signalling in the context of SARS-CoV2 infection.

Prevalence of Metallo-beta-lacatamase in clinical isolates of Pseudomonas aeruginosa and Proteus mirabilis in Benin City, Nigeria.

Olabisi Lawal

University of Benin, Benin City, Nigeria

Olabisi Lawal

Presenter pronouns (eg. she/her, he/him, they/them)
She/her

Abstract

Carbapenems are the prime choice of treatment for severe cases of infections caused by Multi-Drug-Resistant Pseudomonas aeruginosa and Proteus mirabilis. Nevertheless, Metallo-Beta-Lactamase (MBL) production by these organisms has led to carbapenem resistance which is a global threat. This study aimed to determine the prevalence and antimicrobial susceptibility profile of MBL in clinical isolates of Pseudomonas aeruginosa and Proteus mirabilis in Benin City, Nigeria. 354 non monotonous clinical isolates of Pseudomonas aeruginosa (282) and Proteus mirabilis (72) used were obtained from various clinical samples from tertiary hospitals in Benin City. Identification of these isolates were done using standard microbiological techniques. Antimicrobial susceptibility test was performed using Kirby-Bauer disk diffusion method. MBL production was detected using Imipenem Ethylene-Diamine-Tetra-Acetic Acid combined disc test method. Of the total 354 clinical isolates tested, 115 (32.48%) were MBL and the prevalence of this resistant isolates was significantly higher in Pseudomonas aeruginosa (46.8%) compared to Proteus mirabilis (P=0.0001). Among the metallo-beta-lactamase producing isolates of Pseudomonas aeruginosa higher prevalence were reported from Pleural fluid and cerebrospinal fluid samples with 6 (100%) and 3 (100%) respectively. This difference was statistically significant (P=0.0001). Susceptibility testing showed that isolates that produced beta-lactamase demonstrated poorly against cephalosporin, amoxicillin-clavulanate, gentamicin and floroquinones than non-beta- lactamase producers. A prevalence of 46.8% was reported for MBL producing Pseudomonas aeruginosa and 12.5% was reported for MBL producing Proteus mirabilis. Isolates that produced the MBL enzyme were more resistant to antibacterial agents. Measures to control and curb the spread of MBL producing clinical isolates are highly advocated

Investigating the impact of hydration on the dispersal of respiratory droplets and aerosols by healthy study participants

Ailbhe Barry, Nicola Yaxley, Patricia Barkoci, Ginny Moore

UK Health Security Agency, Salisbury, United Kingdom

Ailbhe Barry

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

Respiratory pathogens can be transmitted via droplets and aerosols that are expelled by an individual during respiratory activities such as talking, coughing, sneezing, shouting, and sneezing. To better understand the factors that influence such dispersal, a purpose-built flexible film isolator was used to investigate the generation and dispersal of respiratory droplets and aerosols by healthy individuals. Respiratory bacteria were used as marker organisms for respiratory pathogens and the impact of drinking (hydration) was assessed.

Healthy volunteers (n=10) shouted from one to one hundred (achieving 90-100 dB) prior to, and following, the consumption of 250mls of water. Bacteria-laden droplets (>5 µm) were deposited on settle plates up to 0.5m from source and smaller airborne particles (<5 µm) were captured by air samplers (Anderson sampler and slit-to-agar sampler) situated 1.2m from source. The number of respiratory bacteria dispersed (identified using MALDI-ToF) was compared to determine the impact of hydration.

Considerable interindividual variation was observed among participants. Respiratory bacteria recovered from the settle plates ranged from 24 to 410 CFU before hydration and 36 to 610 CFU after fluid intake when male participants generated significantly more respiratory droplets than females. Fewer aerosolised particles were recovered following hydration. However, a significant (linear) association between the concentration of bacteria in the air and the number deposited on the surfaces was observed. This correlation was not detected before hydration - suggesting that during or immediately after drinking, those individuals who disperse droplets more readily are more likely to generate a higher aerosol concentration.

HIV-1 Virus-Like Particle Release and Quantification

Rowan Casey, Nathalie Signoret

University of York, York, United Kingdom

Rowan Casey

Presenter pronouns (eg. she/her, he/him, they/them)
They/them

Abstract

As part of viral entry, HIV-1 must bind to a coreceptor (commonly CCR5) via its Env complex by its gp120 protein. Studying this interaction cannot be adequately researched by applying virions because post-entry effects affect CCR5, complicating the study of this interaction. Instead, virus-like particles, HIV-1 particles lacking the genome and most of the viral proteins, can be used as a platform for Env to study this interaction without having post-entry effects. The problem with these virus-like particles is that methods to quantify them are time-consuming and expensive as these particles are non-replicative. To overcome this problem, we first optimised the release of HIV-1 virus-like particles containing Env from transfections to provide high titre samples using flow cytometry. Then, we optimised flow virometry for the quantification of these particles. Flow virometry is a relatively new technique that applies flow cytometry to viruses and has seen previous success with both virus-like particles and HIV-1. During these optimisations, we found a role for Env in increasing the localisation of the HIV-1 structural component, Gag, to the plasma membrane. Having completed this project, we have optimised both transfection and flow virometry for the release and quantification of high titre samples of HIV-1 virus-like particles containing Env. In the future we will be using these methods to produce samples for studying the interaction of Env's gp120 with host CCR5.

Aurodox selectively inhibits Type III Secretion in *Salmonella* Typhimurium

David Mark[1], Nicky O'Boyle[2,1], Kabo Wale[1], Rebecca McHugh[1], Andrew Roe[1]

[1]University of Glasgow, Glasgow, United Kingdom. [2]University College Cork, Cork, Ireland

David Mark

Presenter pronouns (eg. she/her, he/him, they/them)
he/him

Abstract

To overcome the looming threat of antimicrobial resistance, novel treatment avenues must be pursued. One of these avenues is the development of antivirulence compounds. By inhibiting pathogen virulence instead of outright killing said pathogen, antivirulence compounds offer several advantages over traditional antibiotics such as a reduced selective pressure to drive resistance. One such compound is aurodox, which has previously been shown to inhibit the locus of enterocyte effacement (LEE) encoded type III secretion system (T3SS) of enterohaemorrhagic *E. coli*, inhibiting the organisms' virulence without killing it or inducing toxin expression. However, its antivirulence effect on other bacteria remains to be explored.

Here we show the effect of aurodox on the SPI-1 and SPI-2 T3SSes of *Salmonella* Typhimurium, . RT-qPCR showed downregulation of the SPI-2 effector *sseB* but not the SPI-1 effector *sipC*. GFP transcriptional reporter assays suggested that aurodox prevents induction of SPI-2, but not SPI-1, based on reduced transcription from the *ssaG* promoter but not the *prgH* promoter. RNA-Seq in SPI-2 inducing conditions ± aurodox showed reduced transcription of 338 genes and increased transcription of 238 genes (4.95 % and 7.03 % of the genome, respectively) – including downregulation of the SPI-2 pathogenicity island and plasmid-borne SPI-2 effectors. Further, aurodox treated *S.* Typhimurium were less able to infect RAW 246.7 macrophage than untreated bacteria.

Taken together, these results show surprise show surprising T3SS specificity in the mechanism of action of aurodox. How this precision is achieved remains to be seen, and raises questions about how aurodox and other antivirulence compounds work on a molecular level.

Investigating the antiviral activity of natural product derived compounds and synthetic xanthones against coronaviruses

Gemma Cooper[1], Bethanie Dean[2,3], Timothy J. Snape[4], Maitreyi Shivkumar[2]

[1]Leicester School of Pharmacy, De Montfort University, Leicester, United Kingdom. [2]Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom. [3]Present address: Department of Chemistry, University of Warwick, Warwick, United Kingdom. [4]Faculty of Health and Life Sciences, De Montfort University,, Leicester, United Kingdom

Gemma Cooper

Presenter pronouns (eg. she/her, he/him, they/them)
She/Her

Abstract

In the last 20 years three pandemic coronaviruses have emerged, with many more coronaviruses circulating in animal reservoirs, future spillover into humans is likely. The development of novel pan-coronavirus antivirals is vital to target new emerging coronaviruses and enhance future pandemic preparedness. Here, we investigated the antiviral activity of a panel of natural product compounds derived from Swertia chirayita for their anti-coronavirus activities.

A panel of compounds and synthetic xanthones based on Swerita bioactive compounds was assessed for antiviral activity against human coronavirus OC43 and 229E, and SARS-CoV-2 pseudoviruses. Infected cell lines were treated with the compounds, and impact on infectivity assessed by quantifying viral titres. Alongside, cytotoxicity of the compounds was determined.

The initial screen identified six natural product compounds that display antiviral activity at non-cytotoxic concentrations. While the synthesised xanthones show a significant reduction in viral infectivity, the inhibition was not as potent as seen in the natural xanthone mangiferin. This suggests that the additional complexity of mangiferin and other naturally derived xanthones may contribute to their increased antiviral activities. Our preliminary data investigating the mechanism of action suggests that the hit compounds inhibit the virus at early stages in the viral lifecycle.

The six compounds identified are being carried forward for further investigations. With mechanism of action studies and viral target determination currently ongoing, this work is a step towards identifying novel pan-coronavirus antivirals.

The effects of nitrate on the oral microbiome: a systematic review investigating prebiotic potential

Siobhan Moran

University of the West of Scotland, Glasgow, United Kingdom

Siobhan Moran

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

Abstract

Nitrate has been identified as a prebiotic for oral health. Evidence suggests dietary nitrate and nitrate supplements can increase the proportion of bacterial species associated with positive oral health whilst reducing species implicated in oral disease(s). In contrast, chlorhexidine-containing mouthwashes commonly used to treat oral infections promote dysbiosis and could be contributing to antimicrobial resistance. Thus, a review of the literature was undertaken surrounding the effects of dietary nitrate on the oral microbiota. Overall, n=15 in vivo and ex vivo studies found acute and chronic nitrate exposure increased facultative anaerobic health-associated Neisseria and Rothia genera (60% and 47% of studies, respectively) whilst reducing disease-associated Prevotella, Streptococcus and Veillonella spp. (33%, 27% and 27% of studies, respectively). Additionally, nitrate altered oral microbiome metabolism, resulting in an increase of pH levels (n=5), which is beneficial to limit oral acidification and caries development. Secondary findings reported the positive effects of nitrate on the systemic system, including cardioprotective benefits (n=5). More clinical trials are required to elucidate the impact of nitrate and its metabolites on oral communities. However, evidence in this review supports the hypothesis that nitrate could be used as a prebiotic for oral health. Future studies should investigate whether chlorhexidine-containing mouth rinses could be replaced by prebiotic treatments.

FLASH POSTERS

Development of a PCR Method for the Detection of Microorganisms Associated with Soft Drink and Juice Spoilage

Samuel Chapman

Teeside University, Middlesbrough, United Kingdom

Abstract

Conventional industry methods for the enumeration of spoilage micro-organisms in soft drinks and juices are often time consuming and resource intensive. A new PCR protocol has been developed for the detection of target dikaryotic fungi and acidophilic bacteria associated with spoilage in soft drinks and juices. It has been demonstrated using in-silico validation and through gradient PCR experiments that two sets of bacterial primers can specifically detect sequences related to acetic acid and lactic acid bacteria – many of these prime agents of spoilage in soft drinks. Fungi primers were modified to allow for a wider coverage of dikaryotic yeasts and moulds relevant to spoilage, however, after successful in-silico validation the primers had limited ability to detect DNA extracts from target yeast and mould reference cultures. Detection of the mould was improved after the addition of magnesium chloride, but increased interfering fragment production. The findings in this work present the initial steps towards an industry-applicable method that could be used as an alternative to conventional microbial enumeration techniques. The food industry is often a "final-thought" sector of technological advancement in STEM, however, integrating a molecular method into beverage manufacturing could product the tangible benefits of reducing product release lead times, optimising technical resource, and providing an environmentally-friendlier alternative to current food-industry lab practices.

Measuring Cytotoxicity in ASFV Infected Cells

Sian Wells[1,2], Chris Netherton[1], Christine Rollier[2]

[1]The Pirbright Institute, Woking, United Kingdom. [2]University of Surrey, Guildford, United Kingdom

Abstract

African swine fever virus (ASFV) is a large DNA virus that causes African swine fever, a haemorrhagic disease of pigs with up to 100% mortality. Due to the virus' size and complexity, the functions of many of the proteins remain unknown. In vitro infection of cells with ASFV usually results in cell death within 24 hours. In vivo other factors, such as anti-ASFV antibody functions, may influence the extent of cell death or the rate at which these cells die. To determine how best to investigate these functions, a variety of cytotoxicity assays were compared for their use to measure cytotoxicity in ASFV-infected cells under laboratory conditions. Here we show that two assays that require transport of components across the cell membrane fail to detect death of ASFV-infected cells, however an assay based on changes of the external cell surface detect cell death successfully. This result suggests ASFV-infected cells undergo membrane changes that interfere with the transport of these components across the cell membrane. This has important implications for the development of assays based on cytotoxicity, such as those determining antibody functions.

Infection status of intestinal parasites in Livestock Slaughtered at the Kumasi Abattoir and the Knowledge and risk among abattoir workers

Seth Offei Addo[1,2], Jennifer Oppong[2], Emmanuel Osei-Frempong[2], Margaret Sena Akpo[2], Godwin Kanfrah[2], John Asiedu Larbi[2]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana. [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Emmanuel Osei-Frempong

Presenter pronouns (eg. she/her, he/him, they/them)
he/him

Abstract

This study aimed at investigating the prevalence of gastrointestinal parasites in the livestock slaughtered at the Kumasi abattoir and further determine the level of awareness of the abattoir workers on the risk of zoonosis. A total of 150 faecal samples were collected from goats, cattle, sheep, pigs and examined using both the Direct smear (DS) and the Formol-ether concentration (FEC) methods. It was observed that the prevalence of intestinal parasites in the collected samples using the FEC method was 25.3% as compared to 19.3% using the DS method. With the FEC method, it was observed that Hookworm (14.7%), Ascaris sp (8.7%) and Balantidium coli (2.7%) occurred in all the sampled animals. Furthermore, ten different intestinal parasites were identified using the FEC method whiles eight were identified using the DS method. Thus, the FEC method was found to be more efficient for intestinal parasite detection in livestock faecal samples. From the study, it became evident that a significant number of abattoir workers were unaware of parasitic infections (51.2%) or the potential zoonotic transmission of intestinal parasites (52.5%). Considering the number of gastrointestinal parasites recovered and the limited knowledge of the abattoir workers in the study area, it is essential to encourage regular deworming of the abattoir workers as well as to conduct routine health education in the abattoir to prevent the spread of infections.

Investigating the *Bacillus subtilis* soil isolate response to growth in complex media

Amy Wood, Nicola Stanley-Wall, Thibault Rosazza

University of Dundee, Dundee, United Kingdom

Amy Wood

Presenter pronouns (eg. she/her, he/him, they/them)
She/her

Abstract

Bacillus subtilis is a Gram-positive bacterium that can be isolated from many environments including plant root surfaces. It produces eight extracellular proteases, and recent work has shown that collectively they support growth when the sole nutrient source is in the form of a polymeric protein. Our hypothesis is that recently isolated *B. subtilis* strains may be better adapted to grow on complex nutrient sources, as these conditions are likely to be encountered in the natural environment. A library of sequenced *B. subtilis* isolates, that exhibit a high degree of genomic diversity, was available to test our hypothesis. It was first determined that soil isolates used in the analysis all have the genes encoding the eight extracellular proteases. Next, we determined that soil isolates could grow using a polymeric nutrient source, however, the growth dynamics were found to differ depending on the isolate's phylogenetic relatedness to the laboratory progenitor strain, NCIB 3610. It was established that there was a direct correlation between the growth dynamics displayed by the isolates in a polymeric nutrient source and the level of extracellular protease production. In future work, by the construction of genome deletion(s) in the peptide ABC-importers, we intend to determine if they play a fundamental role in the support of growth on a polymeric nutrient source. We are also exploring the underpinning regulation mediating the differences in growth.

Standardising a method to investigate the transfer of microorganisms between Artificial Finger Pads and test surfaces.

Martina Williams[1], Simon Parks[1], Jack Vincent[2], Maurice Walker[2], Richard Thomas[2], Beth Farrow[2], Merlin Etzold[2], Ailbhe Barry[2], Natalie Brown[3], Alexander White[1], Thomas Pottage[1], Ginny Moore[1], Paz AranegaBou[1]

[1]UKHSA, Salisbury, United Kingdom. [2]Dstl, Salisbury, United Kingdom. [3]University of Surrey, Guildford, United Kingdom

Martina Williams

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

High-contact surfaces can become contaminated with potential pathogens and, in the absence of effective cleaning, can act as fomites and spread infection. Whilst previous studies have investigated fomite transmission differing experimental protocols have produced variable results, leading to a need for a standardised method for investigating the transfer of microorganisms between hands and surfaces.

A method to inoculate test surfaces (coupons) with microorganisms deposited as a dry aerosol (<2 micron, RH ranging from 4.6- 34.7 %) was developed. Using this method, stainless steel coupons were inoculated with Bacillus atrophaeus spores and touched using an Artificial Finger Pad (AFP). AFPs were modelled using the index finger of a-31-year-old female and were mounted in a contact rig that simulates touch with a force of 15N for 1 second contact time. To validate this technique, two operators performed one contact event across three independent experiments.

Contact between AFPs and stainless-steel coupons inoculated with B. atrophaeus (aerosolised from sterile water) resulted in a mean transfer efficiency (n=6) of 45 ± 18% (average ambient RH during contact of 42.0%). The transfer efficiency did not significantly differ between three independent experiments or between the two operators of the rig, demonstrating the consistency of the methods used to inoculate the coupons and to assess the transfer.

The experimental set up simulates the deposition of contaminants and subsequent transfer during touch. Future work will investigate transfer efficiencies for other microorganisms and surface materials.

Characterisation of Staphylococcus epidermidis NCIMB 8558

Emily Horsburgh[1], Elisa Caberlotto[2], Massimo Vassalli[1], Andrew Roe[1]

[1]Glasgow University, Glasgow, United Kingdom. [2]L'Oreal Paris, Paris, France

Abstract

Staphylococcus epidermidis is a natural coloniser of the skin microbiome and is usually considered a commensal organism. Commensal strains such as S. epidermidis are currently being considered for numerous bioengineering applications in a living biomaterial. These living biomaterials are a novel approach to biomedical applications such as topical delivery systems as well as industrial uses which are more sustainable than current methods. However, to manipulate S. epidermidis strains, knowledge of their restriction-modification (RM) systems and any acquired antibiotic resistance mechanisms is key. We used Illumina sequencing to sequence the S. epidermidis NCMIB 8558 genome, which was not publicly available at the time. Illumina sequencing showed that the complete genome of S. epidermidis 8558 consists of 2.3 Mbp. We assembled the sequence and annotated different aspects of the genome, for example RM systems and antibiotic resistance genes using the bioinformatic tools HMMR and CARD, respectively. In addition, we performed evaluation of 16S ribosomal RNA to determine this strain's phylogenetics and performed a comparative study against other S. epidermidis strains. Furthermore, pan-genome studies were carried out with our newly characterised 8558 strain with a mixture of fully sequenced clinical and commensal isolates to understand the differences between the strains. This study determined the ability to progress with S. epidermidis NCMIB 8558 for further testing.

The effects of herbal teas and supplements on the human microbiota and metabolism

Oluwadamilola Okeyoyin[1], Emmanuel Adukwu[1], Oliver Gould[1], Vivien Rolfe[2]

[1]University of the West of England Bristol, Bristol, United Kingdom. [2]Pukka Herbs, Bristol, United Kingdom

Oluwadamilola Okeyoyin

Presenter pronouns (eg. she/her, he/him, they/them)
She/Her

Abstract

Background: For centuries, herbal teas have been used for therapy in different civilisations and medical systems such as the Chinese, Indian, indigenous, and African. It is believed that bioactive compounds-phytochemicals contained in herbal teas have a wide range of biological effects. Research show that herbal teas can contribute to health via the microbial flora which is found majorly in the gut and oral cavity. However, the mechanisms by which they contribute to health and wellness is not well established.

Aim: The aim of this research is to determine the effects of selected herbal teas on oral and gut bacterial species and their mechanisms of action.

Methodology: The herbal blends investigated include mint, fennel, ginger, and lemongrass. The bacterial species investigated include Streptococcus mutans, Streptococcus pyogenes, and Escherichia coli. Head Space Gas Chromatography Mass Spectrometry (HS-GC-MS) was used to determine the phytochemical composition of the steam of the herbal brews and the antimicrobial effects of the herbal teas was determined using disk diffusion and microbroth dilution assays.

Results: This research shows that the herbal teas have extensive volatile phytochemical profiles. The most abundant VOCs were Menthol in the mint tea, eucalyptol in the ginger tea and anethole in the fennel tea. Furthermore, the herbal teas showed some antimicrobial activity on the microorganisms investigated.

Conclusion: Herbal teas do have inhibitory effects on the growth of certain bacterial species however, further research is required to determine the mechanism by which they exert these functions.

POSTERS

The Role of IgA in Protection against Microbial Keratitis:investigation of the effect of secretory IgA from milk using human and porcine models

Waad Aljohani

University of sheffield, sheffield, United Kingdom

Abstract

Infection of the cornea (microbial keratitis) is a major problem in less developed countries that if treated incorrectly can lead to corneal blindness an immortalised porcine epithelial cell line (derived from porcine intestine, IPEC-J2, is available and we have also used primary porcine corneal epithelial cells (PCEipC). IgA or menschen IgA is an immunoglobulin, which are considered the first defense line that triggers resistance in cows against microbial keratitis infection. The IgA works through inhibiting the bacterial pathogens causing microbial keratitis from adhering to the epithelial cells. In the protection against microbial keratitis, IgA plays an essential role involving inhibiting the bacterial pathogens, which causes the popular microbial keratitis from binding to the epithelial cells in a cow. Pre-treatment of IPEC-J2 epithelial cells with immunoglobulin IgA from (UK) decreased adhesion of S.aureus to these cells, whilst there was as well effect on adhesion to primary epithelial cells . In summary, it appears that immunoglobulin IgA from (UK) may be involved in adhesion of S.aureus to porcine intestinal epithelial cells, and porcine corneal epithelial cells. The porcine corneal microbial keratitis model is therefore likely to be suitable for immunoglobulin IgA from (UK) and useful for inhibitors of bacterial adhesion demonstrated efficient prevention of bacterial adhesion to both IPEC-J2 and PCEipC cells by IgA.

Efficacy of Handwashing Techniques in Controlling the Spread of *Staphylococcus epidermis* in Healthcare Settings

Sevcan Gazi

London Metropolitan University, London, United Kingdom

Sevcan Gazi

Presenter pronouns (eg. she/her, he/him, they/them)
she/her

Abstract

The increased incidence of microbial contamination alongside transmission of pathogens from one individual to another has been established to be majorly through the hands of healthcare workers. Proper hand hygiene measures can prevent the transmission of pathogens, such as *Staphylococcus epidermidis.* Therefore, this study aims to analyse the efficacy of various hand hygiene techniques and antiseptic agents to reduce the transmission of *Staphylococcus epidermidis*. Using the spread plate method, participants' hand gloves infected with *Staphylococcus epidermidis*, as well as those labelled "prior to washing," with "hand soap washing," and "antiseptic washing" were swabbed on agar plates and incubated at 37°C for 24-48 hours. After incubation, the plate was examined for bacterial growth, and the significance of hand washing was analysed. Results indicate that the agar plate labelled "prior to washing" showed the highest bacterial growth, indicating that the hands had the highest concentration of bacteria before washing. The agar plate labelled "hand soap washing" showed a reduced amount of bacterial growth, indicating that handwashing with soap was effective in reducing bacterial transmission. The agar plate labelled "antiseptic washing" showed the least amount of bacterial growth, indicating that antiseptic gel was the most effective method of hand hygiene in reducing bacterial transmission. The results of this experiment suggest that hand washing with soap and water, along with the use of antiseptic agents, is effective in reducing the transmission of *Staphylococcus epidermidis*. Thus, using the most effective hand hygiene procedures can reduce healthcare-associated infections and enhance patient outcomes.

Investigating the biofilm-forming ability of *Staphylococcus capitis* NCRS-A versus non-NRCS-A strains via microtiter assay.

Alexander White, Laura Steege, Ailbhe Barry, Ginny Moore

UK Health Security Agency, Salisbury, United Kingdom

Alexander White

Presenter pronouns (eg. she/her, he/him, they/them)
He/Him

Abstract

A multi-drug resistant clone (NRCS-A) of *Staphylococcus capitis* has been detected worldwide in multiple neonatal intensive care units (NICU) and has become increasingly associated with late-onset neonatal sepsis. *S. capitis* NRCS-A has been shown to persist within the NICU environment but reasons for this are unknown. Bacterial attachment to surfaces can increase their tolerance to disinfectants. This study investigated the ability of different strains of *S. capitis* (NRCS-A strains and non-NRCS-A strains) to attach to surfaces.

Using a microtiter assay with crystal violet staining, eight *S. capitis* strains, representing NRCS-A strains and non-NRCS-A strains, were assessed for hydrated biofilm production alongside other species under different nutrient concentrations, and plate treatment types. Other organisms tested included *Pseudomonas* spp and other staphylococci, comprising clinical and environmental strains.

Pseudomonas strains showed strong biofilm formation on both plate types. The attachment of *S. capitis* differed depending on plate surface. NRCS-A strains attached significantly better to treated surfaces than to non-treated plates ($P < 0.05$) while the attachment of non-NRCS-A strains was not significantly affected ($P > 0.05$). Nutrient concentration also appeared to impact the attachment of NRCS-A strains ($P < 0.05$). However, in comparison to other staphylococci (e.g. *S. warneri*), *S. capitis* attached less readily to surfaces.

S. capitis, including NRCS-A strains, does not readily attach to surfaces. However, surface material may facilitate attachment of the NRCS-A clone. Future work will focus on different surface types and the ability of *S. capitis* to produce dry surface biofilm that contain limited nutrients and moisture compared to 'hydrated' biofilms.

Generating Tuneable Mycelial Networks for Directed Assembly

Dooshima Nevkaa, Carole Perry

Nottingham Trent University, Nottingham, United Kingdom

Dooshima Nevkaa

Presenter pronouns (eg. she/her, he/him, they/them)
She/her

Abstract

In our search to understand the effects of environmental changes on the structure and Biochemistry of fungi, a range of fungal species have been cultured in liquid (Potato Dextrose Broth) and solid (Potato Dextrose Agar) media under varying and controlled environmental conditions. The growth, structure, and morphology of the fungal mycelia mat has been studied extensively. We have also studied the biochemistry of the key components (chitin, carbohydrates, and proteins). These components have been identified and characterised. In addition, we also observed effect of temperature change and an additional glucose (in the media) on the on the mycelial Physical Properties. Our hypothesis is that by understanding how the local environment affects chemical synthesis and growth patterns we will be able to tailor the structure of functional composite materials based on fungal mycelia.

Methodological Considerations in Characterising the Microbiome of Semen.

Richard Stack

University of Kent, Canterbury, United Kingdom

Richard Stack

Presenter pronouns (eg. she/her, he/him, they/them)
He/him

Abstract

The relationships between the microbiome of the male reproductive tract, semen quality and fertility metrics, remain poorly defined. Clustering of community state types is inconsistent between research groups and a clear characterisation of dysbiosis is elusive. Whilst high-throughput sequencing technologies have allowed for resolution of previously unculturable microbes, the sensitivity of these techniques create multiple opportunities for bias.

In an attempt to test our sequencing pipeline for bias, we included a mock microbial community. By combining axenic cultures isolated from boar semen we created a dataset that, whilst idealised, carried relevance to the *in-vivo* microbial community in terms of phylogenetic representation. Individual mock community members provided data on the effectiveness of lysis and DNA extraction, which we optimised for this unusual sample type. The mock community also allowed for identification of false positive sequences. This included sequences at high abundance indicating presence of contaminants (e.g. *Ralstonia*), and those at low abundance, potentially sequencing artefacts, informing the definition of a filtering threshold in subsequent data analysis. Finally, seeding semen samples with the mock community, resulted in failure to amplify certain community members, indicating potential inhibitors to lysis, extraction or PCR amplification inherent within semen itself. In deconstructing these biases, we aim to characterise seminal microbiota with confidence and hope to uncover their relationship to fertility.

Characterisation of stable and extreme (800kb) copy number variation in *Bordetella pertussis*

Sarah Cameron[1,2], Jonathan Abrahams[3], Dorothy Tam[1,2], Joss Etty[1,2], Mikey Ingrouille[1], Andrew Preston[1,2]

[1]University of Bath, Bath, United Kingdom. [2]Milner Centre for Evolution, Bath, United Kingdom. [3]Oxford Nanopore, Oxford, United Kingdom

Sarah Cameron

Presenter pronouns (eg. she/her, he/him, they/them)
She/her

Abstract

Tandem duplication or copy number variation (CNV) in bacteria is largely under studied due to the unstable nature and often intractable size of the resulting genomic structures. However, it is thought that CNV is a common method of adaptation among bacteria, allowing for selection of phenotypes including increased virulence and heteroresistance (AMR). Yet comprehensive studies and characterisation of these amplifications are missing. Here we have isolated an extreme >800kb CNV in a single clone of the UK54 strain of *Bordetella pertussis*, which remarkably appears to have no effect on growth, and is stable enough to be studied. Initial analysis by qPCR suggested this 16kb region has a copy number of 51.21. This has been further supported by visualisation of the full duplication using pulse field gel electrophoresis, a correlation of mRNA transcript levels to copy number using RT-qPCR and using the read depth from ultra-long sequencing reads (Oxford Nanopore) as a proxy for copy number. Surprisingly growth assays show no significant difference between either the wild-type, or another clone of UK54 with a lower copy number of 4.1. Therefore, implying that this clone is carrying an extra 20% of its genome with no apparent fitness cost or benefit *in vitro*. This observation demonstrates that *B. pertussis* is a suitable model organism for studying copy number variation and demonstrates that large genome duplications can be captured for further study and characterisation.