Abstracts book

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Typing of Salmonella species Prevalent Among Children Having Diarrhoea in Parts of North-Western Nigeria

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Abstract

In recent times, problems related to incidence and severity of *Salmonella* infections have increased significantly. To determine the *Salmonella* serotypes affecting children with diarrhoea in North-western Nigeria, a total of 634 stool samples of children aged five years and below, presenting with diarrhoea were collected and investigated for *Salmonella* infection by culture, biochemical and serology. The 16s ribosomal RNA was sequenced for each isolate to determine specific serotypes involved in the infection. The overall prevalence of *Salmonella* species among the study subjects was 4.1%. The highest prevalence occurred among children aged 25-36 months (10.4%). Sequence analysis of the 16S rRNA obtained from the isolates showed that of 18 isolates sequenced,11(61.1%) were affiliated to *Salmonella enterica* subspecies enterica serovar Typhi CT18, while 7(38.9%) were affiliated to *Salmonella enterica* subspecies enterica serovar Typhimurium LT2. The percentage identity for all the 16S sequences ranged between 86 - 99%, with E-values of zero in all the isolates except one. *Salmonella* still remains one of the major and most important bacterial pathogen of diarrhoea among children in the study area. Provision of adequate potable water and careful surveillance and monitoring of incidence and spread of diarrhoeal diseases, may help to reduce the disease burden in children.

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Isolation and characterization of novel bioactive compounds from marine rare actinomycetes

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Abstract

One of the global challenges of the current decade is antimicrobial resistance. This era calls for new approaches in antimicrobial drug discovery which includes, a continuous search for antimicrobials that are safe, efficient and effective against pathogens including multi-drug resistances ones. Continuous and repeated isolation of actinomycetes from terrestrial environments have limited the discovery rate of new bioactive compounds against resistant pathogens. It has been postulated that the unexplored and underexplored habitats including the marine habitat could be a very rich source for the isolation of rare actinomycetes, with the potential to produce novel bioactive compounds. The aim of this project is to isolate, characterize and determine novel bioactive compounds from marine rare *actinomycetes*. To achieve this, samples (clear water, sediment, and green lichen) were collected from Liverpool Sea, UK. The samples were pre-treated, vortexed and differentially centrifuged. Standard cultural-based techniques were used in the inoculation, culturation and selective isolation with special enriched media. Standard molecular biology techniques such as DNA extraction, 16s rDNA amplification, DNA purification, gel electrophoresis, sequencing, and NCBI nucleotide blast were carried out on the isolates. The 16S rRNA gene sequences revealed that the isolates (twelve) were phylogenetically related to the *Alteromonas*, *Pseudomonas*, *Brevibacillus*, *Sphingobacterium*, *Marinobacter*, *Paenibacillus* genera. These isolates have given an insight into the biodiversity of the marine ecosystem and show that the Liverpool sea as a promising source for the isolation of marine organism (actinomycetes). This habitat can be exploited for novel bioactive compounds with biotechnological and pharmaceutical potentials.

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Biomaterial Modulation for Management of Catheter-Associated Urinary Tract Infection (CAUTI)

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Abstract

Healthcare acquired infections (HCAI) occur in 7-10% of all hospital admissions, with indwelling medical devices such as urinary catheters frequently being associated as the origin of the infection.
Silicone is often used as the biomaterial used in the manufacture of medical devices and therefore, incorporating an antimicrobial component to this material could inhibit development of biofilms and thus reduce HCAI occurrence.
Minimum inhibitory concentrations (MICs) of triclosan plus 9 novel compounds were established using broth microdilution methods against 7 bacterial species and the fungus *Candida albicans*. Minimum biocidal concentrations (MBCs) were also determined by culture on agar following MIC assessment. Antibiofilm assessment involved exposing preformed biofilms in 96-well plates to the antimicrobials. Regrowth of biofilm post treatment was determined by optical density.
Lead compounds were selected and incorporated into silicone materials and antimicrobial activity of the biomaterial was evaluated via zone of inhibition assays and quantification of biofilm formation on silicone surfaces by live/dead staining and confocal laser scanning microscopy.
Antimicrobial silicone containing 1% triclosan was effective against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Providencia stuartii*. The material was not antimicrobial against *Serratia marcescens* or *Pseudomonas aeruginosa*.
Silicone coated with an acetoxy coating containing a novel imidazolium compound produced larger zones of inhibition than non-coated silicone against *Candida albicans*.

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Antibiogram and beta lactamase genes among cefotaxime resistant E. coli from wastewater treatment plant

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Abstract

The World Health Organization (WHO) recently classified Enterobacteriaceae resistance to third-generation cephalosporin into the group of pathogens that encompasses critical criteria for future research. A study to assess the antibiogram and beta-lactamase genes among the cefotaxime resistant E coli (CREc) from a South African wastewater treatment plant (WWTP) was conducted using standard phenotypic and molecular biology characterization methods. An approximate total *E coli* (TEc) concentration (log_{10}CFU/mL) ranged between 5.7 - 6.8 among which cefotaxime resistant *E coli* were between 1.8 - 5.2 (log_{10} CFU/mL) for cefotaxime antibiotic concentration of between 4 - 8 mg/L in the influent samples. Effluent samples were heavily influenced by the chlorination and had only 0.3 log10CFU/mL of TEc. Fifty-one of a total of 75 selected cefotaxime resistant isolates were further subjected to new round testing, with a follow up of 36 and 48 isolates for both colistin and gentamycin respectively, guided by original results. Selected CREc exhibited resistance to amoxicillin-clavulanic acid (35.3 %; n=51), colistin sulphate (76.5 %; n=36), ciprofloxacin (47.1%; n=51), gentamycin (87.5%; n=48) and intermediate-resistance to meropenem (11.8%; n=51). Extended spectrum-beta lactamase genes, *bla*$_{CTX\text{-}M}$ (52.6%) and *bla*$_{TEM}$ (84.2 %) and concurrent *bla*$_{CTX\text{-}M}$+*bla*$_{TEM}$ (36.8 %) were detected, but no

bla$_{SHV}$. Carbapenem resistance genes, *bla*$_{KPC-2}$ (15.8 %), *bla*$_{OXA-1}$ (57.9 %), *bla*$_{NDM-1}$ (15.8 %) were also detected. Approximately, 10.5 % - 36.8 % co-occurrence of two or beta-lactamase genes was detected in some isolates. Resistance to cefotaxime and the presence of wide range of beta-lactamase genes showed the potential risks associated with these pathogens occupational exposure

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Lignocellulose digestion by anaerobic rumen microbial consortia from sheep

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Abstract

Rumen microbial community is excessively studied to understand its complex anaerobic microbial interactions. These consortia are not uniform but very diverse. They degrade complex plant biomass into easily metabolizable compounds by cellulolysis. The aim of this study was to enrich anaerobic cellulolytic consortia from sheep rumen fluids and to understand the possible mechanisms of cellulolysis with reference to their lignocellulolytic enzyme production. The rumen fluid samples were enriched in different culture media containing cellulose. The experiment was conducted in an anaerobic glove box with an atmospheric composition of 90% N_2, 5% H_2 and 5% CO_2. After 4 weeks of incubation at 37 ˚C temperature, homogenized cell suspensions were assayed for their total cellulase, xylanase, exoglucanase, endoglucanase and laccase activities. The most efficient cellulolytic enzyme producer-consortium was RF5, producing the highest total cellulase activity of 0.549 FPU/ml, highest xylanase activity of 0.582 U/ml and highest endoglucanases activity of 0.81 U/ml. The exoglucanase activity was 0.0985 U/ml. However, the laccase production of the 28 consortia investigated was negligible and only several consortia were endoglucanase positive. The regression analysis of enzyme activity data revealed that there is a positive correlation between total cellulase, xylanase and exoglucanase activities of consortia investigated. This reveals that the changes in total cellulase activity might affect the expression of xylanase and exoglucanase. These consortia will mainly release xylose, cellobiose and glucose from lignocellulose. Moreover, RF5 being the most efficient mesophilic anaerobic consortium among investigated consortia should have a multicomponent cellulosome with xylanase, endoglucanase and exoglucanase.

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Characterization of the gamma-glutamylpolyamine synthetase GlnA3 in *Mycobacterium tuberculosi*s as a potential drug target

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Abstract

Human intracellular pathogenic actinobacterium *Mycobacterium tuberculosis* has developed strategies to access nutrients from the host and to exploit the host to synthesize more resources for its growth and propagation. *Mycobacterium tuberculosis* can induce the polyamine biosynthesis during the shift in metabolic state of macrophages. The pathogen is able to utilize polyamines as a sole *N*- and *C*-source to support its own intracellular growth in macrophages. In our previous studies in a model actinobacterium *Streptomyces coelicolor* M145, we demonstrated that a protein annotated as glutamine synthetase-like, GlnA3$_{St}$ (SCO6962), is involved in the first step of polyamine utilization pathway[1]. GlnA3$_{St}$ is a gamma-glutamylpolyamine synthetase (GPS) that ensures both nutrients availability (*C*- and *N*-source) and resistance against high polyamine concentrations in *Streptomyces coelicolor*[1]. Since there is a homologue of GlnA3$_{Mt}$ (Rv1878) in *Mycobacterium tuberculosis,* this GPS enzyme is a particularly interesting target for drug development. In our current studies we were able to show that GlnA3$_{Mt}$ can glutamylate polyamines, demonstrating GPS activity. Thus, inhibition of GlnA3$_{Mt}$ may

result in the stop of the nutrient flux and subsequent death of the pathogen and might be an effective therapeutic strategy since these kind of enzyme do not naturally occur in eukaryotes.

1. **Krysenko S.**, Okoniewski N., Kulik A., Matthews A., Grimpo J., Wohlleben W. and Bera A. (2017) Gamma-Glutamylpolyamine Synthetase GlnA3 Is Involved in the First Step of Polyamine Degradation Pathway in *Streptomyces coelicolor* M145. *Front Microbiol* **8**: 726. doi: 10.3389/fmicb.2017.00726

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Evaluation of antifungal activities and antihaemolytic effects of Cinnamon essential oils from leaf and bark on *Candida albicans* and *Candida auris*

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Abstract

Candida infections can be considered as a significant source of patient morbidity and mortality. *Candida albicans* is the most common pathogen causing Candida infections. *Candida auris* is a newly described pathogen that is associated with multi-drug resistant candidiasis and candidaemia in humans. The antifungal effects of various essential oils and plant compounds have been demonstrated against human pathogenic fungi. In this study, the effect of Cinnamon leaf and bark essential oil (CEOs) was determined against both *C. albican*s and *C. auris.* The disc diffusion (direct and vapour), minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC) methods were used to determine antifungal activity of the EOs against selected strains (*C. albicans* ATCC 10231, *C. albicans* ATCC 2091 and *C. auris NCPF 8971)* whilst the mode of action and haemolysin activity of the CEOs were determined using electron microscopy and light microscopy.Disc diffusion assays showed the inhibitory activities of bark CEO was higher than leaf CEO in both direct and vapour phase. The MICs and MFCs of bark CEOs for all tested strains was below 0.03%, which was lower than the MICs of the leaf CEO (0.06 – 0.13 % v/v) dependent on the strain and the MFCs at 0.25% v/v. In the morphological interference assays, it was observed that the CEOs damaged the cell membrane and inhibited pseudohyphae formation. The haemolysin production assay showed that CEOs can reduce the haemolytic activity of all tested strains.In conclusion, CEOs showed *in vitro* antifungal and antihaemolytic potential against *C. albicans* and *C. auris.*

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ORAL CANDIDIASIS IN HIV INFECTED PATIENTS VISITING SUKRARAJ TROPICAL AND INFECTIOUS DISEASE HOSPITAL(STIDH), TEKU, KATHMANDU AND ITS ANTIFUNGAL SUSCEPTIBILITY PATTERN BY DISC DIFFUSION METHOD.

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Abstract

The low absolute CD4+ T-lymphocyte count has traditionally been cited as the greatest risk factor for the development of Oral Candidiasis (OC) and current guidelines suggest increased risk once CD4+T-lymphocyte counts fall below 200 cells/mm³. Rampant, indiscriminate and long-term use of antifungals has led to the development of antifungal resistance among Candida species. Hence, a cross sectional study was conducted to isolate, identify and perform antifungal susceptibility testing of *candida* spp.
A total of 408 oral swab samples were collected from patients with and without active lesion visiting ART (Anti-Retroviral Therapy) center of STIDH. Samples were processed on Sabouraud Dextrose Agar (SDA), if any growth was observed, gram staining was performed, and observation of gram-positive yeast cells led to subculture on HiCrome™ Candida Differential Agar for differentiation of *Candida* spp. based on colouration and colony

morphology. Further, identification of species was done based on germ tube test, chlamydospore production on Corn Meal Agar and Sugar fermentation test.
Candida albicans (n=53), *Candida tropicalis* (n=3), *Candida krusei* (n=2), Candida glabrata (n=1), and other *Candida* spp. (n=6) were the organisms isolated. There was a significant association between oral Candida carriage and CD4+ cell count ≤ 200 cells/mm³ (p<0.001), URTI and oral Candida isolation (p=0.029), recent antibiotic consumption and oral Candida isolation (p=0.002). In contrary, there was no significant association between ART use and oral Candida infections (p=0.188), no association between tobacco consumption and Oral Candida isolation (p=0.051). Amphotericin-B was the most sensitive and Fluconazole the most resistant antifungal among *candida* spp.

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Surveillance of β-lactam, azithromycin and fosfomycin resistance in non-typhoidal *Salmonella:* Characterisation of an *S.* Infantis plasmid

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Abstract
Non-typhoidal *Salmonella* (NTS) infections are associated with high morbidity and mortality. β-lactams are used as first-line treatment but resistance to these has increased considerably in recent years. Azithromycin and fosfomycin are used as alternatives; however, the incidence of resistance in these drugs is also increasing. Epidemiological surveillance on 35,372 NTS received by Public Health England was conducted for analysis of demographics, including global travel. Genomic typing and antimicrobial resistance data for *Salmonella* isolates were used to determine the prevalence of β-lactam, azithromycin and fosfomycin resistance in NTS over a four year period. No isolates were resistant to β-lactams, azithromycin or fosfomycin alone but all isolates were resistant to multiple antimicrobial classes. IncHI2, IncY and IncN plasmids were predominantly found in the most multi-drug resistant isolates. Multi-drug resistance (MDR) was particularly a concern in the *S.* Infantis population. Therefore, long read sequencing was used to characterise an MDR *S.* Infantis isolate. Three drug regions were identified in a IncFIB, a megaplasmid identified in this isolate. The resistance determinants *fosA, arsA, arsD* and $bla_{CTXM65,}$ were discovered on the same drug region. Analysis of IncFIB in this *S.* Infantis isolate revealed 99% similarity to a IncFIB plasmid in *S.* Infantis isolated from chickens in the USA. Horizontal gene transfer of AMR genes is the most likely cause of the increase in resistance genes detected within the *Salmonella* population in both humans and animals. Whole genome sequencing utilised in global surveillance of *Salmonella* isolates allows characterisation of AMR determinants and prediction of emerging resistance profiles in the UK *Salmonella* population.

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Characterising the inhibition profile of a novel antimicrobial

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Abstract
Introduction: Antibiotic resistance is one of the greatest problems facing the 21st century with few new classes of antibiotics being discovered. The forefront of antibiotic discovery has been the soil microbiome and it is still a valuable resource for identifying microbes with possible antibiotic producing capabilities leading to novel classes of antibiotics. This justifies continuing investigation into the soil microbiome for antibiotic producing bacteria, to help tackle the growing trend in antibiotic resistance. A bacterial soil isolate was found

to inhibit *Enterococcus faecalis* ATCC 29212 and the aim of this work was to further characterise the inhibition profile of the antibacterial.
Methods: Bacterial plug and supernatant assays were used to access the inhibition ability of a soil bacterial isolate against the WHO "priority pathogens". Identification of the bacteria was carried out using 16S rRNA and whole genome sequencing. Synergy tests were carried out using broth dilution assays.
Results: The soil isolate (N5) was identified as Enterococcus and showed antibacterial activity against *Staphylococcus aureus* MRSA and *E. faecalis* VanA. This antibacterial is secreted into the supernatant which still showed inhibitory activity against MRSA and VRE. Synergy with N5 and ciprofloxacin (0.2 µg/ml) against *E. faecalis* ATCC 29212 was also observed.
Conclusion: Both VRE and MRSA are important players in nosocomial infections and are displaying high levels of resistance. Further study of this antibacterial could lead to the development of a new compound to help overcome resistance mechanisms or a novel antimicrobial.

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Exploring Coastal Plants as a source of Plant Growth Promoting Endophytic Bacteria

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Abstract
Global soil salinity is rising; it is estimated that 20% of irrigated agricultural land globally is currently contaminated by high salinity levels (>40mM NaCl), with more land contaminated each year. Plant growth promoting bacteria (PGPB) can mitigate the impact this has on growth of agricultural crop plants worldwide, as they can be capable of promoting plant growth and minimising salt stress effects. Plants already living in saline environments are a potential target for discovering novel PGPB for agricultural application. As a less-well-explored bacterial niche, the endosphere of plants represents a potential reservoir of new bacterial strains and species.
Halotolerant bacteria were isolated from the endosphere of plants living in saline coastal environments near Aberystwyth and identified by 16S sequencing. All 61 isolates were screened for halotolerance; all showed rapid growth on salt concentrations up to 5%w/v, while 28 isolates were capable of growth on Nutrient Agar supplemented with 2 Molar NaCl. *Brachypodium distachyon* BD21 plants were inoculated with these isolates and subjected to salt stresses of 0mM, 100mM and 200mM NaCl in a large-scale screen in the National Plant Phenomics Centre in Aberystwyth. Plant growth traits including total biomass and seed production were monitored to screen for growth promotion potential of the endophytes under these conditions. 13 of 61 isolates increased biomass with respect to controls in the absence of salt while 9 increased biomass compared to the controls under salt stress conditions. The isolates were further screened for production of bioactive compounds using methods including GCMS and LTQ-MS/MS.

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Isolation of Bacterial Strains From Compost with Biocontrol Ability Against *Burkholderia glumae* Infection in Rice Seedling
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Abstract
Burkholderia glumae causes seedling rot of rice, an increasingly prominent disease problem in global rice production. Our recent experiment showed adding 10% (w/w) of three types of compost produced by Hazaka system on the soil could suppress the emergence of *B. glumae* infection on the rice seed. We aim to isolate and identify bacterial strain from compost that have a role on compost biocontrol ability to protect rice from

B. glumae infection. Subsequently, we conducted *in planta* assessment of biocontrol activity against *B. glumae*; *in vitro* assessment for antimicrobial activity against *B. glumae*; and quorum quenching assessment using isolated strains. Strains with high biocontrol activity were further subjected to 16S rRNA phylogenetic classification and their abundance in different stage of composting process was investigated by MiSeq sequencing. We isolated 30 bacterial strains. As a result, 7 out of the 30 (23.3%) isolated strains were showed strong biocontrol ability. However, none of the isolated strains were antimicrobial positive, whereas 11 strains were showing quorum quenching positives that might indicating its biocontrol mechanism. Strain Oono 8-d1 that was selected for its highest biocontrol activities was related to *Chryseobacterium vietnamese* at 98.41% by 16S rRNA gene sequence analysis – indicating it as a novel species. MiSeq analysis showed genus *Chryseobacterium* was found as minor bacteria. This result suggested that the compost ability to suppress *B. glumae* infection on rice did not solely depend on *Chryseobacterium*activity and it is further necessary to investigate the unidentified bacteria roles.

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Identification and characterisation of a novel SXT/R391 ICE-like mobile genetic element isolated from an Irish wastewater environment.

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Abstract

Wastewater treatment plants accumulate a large and diverse number of microorganisms including many *Gammaproteobacterial* species. Many of these organisms can harbour antibiotic and heavy metal resistance genes associated with plasmids, transposons and Integrating Conjugating Elements (ICEs). As many ICEs are found in *Gammaproteobacterial* species, this makes wastewater a potential reservoir for ICE elements particularly the SXT/R391 family.

SXT/R391 ICE-like mobile genetic elements are the largest family of ICEs, distinguished by a Type I integrase. The elements integrate into the *prfC* gene of their host, replicate with the chromosome and then transfer via conjugation. This allows the element to be very stable and to be spread widely. The elements share 51 core genes which make the 'backbone', which allows for integration, excision and transfer. SXT/R391 elements contain five hotspots and four variables regions which allow for the insertion of heterologous DNA or gene segments which provide the organism with an adaptive advantage.

We aimed to isolate and characterize an SXT/R391-like ICE from an Irish wastewater treatment environment which has as yet not been reported. Screening via PCR and element specific probes was initially employed to identify an SXT/R391-like ICE from wastewater using enrichment on selective media for Gammaproteobacteria. A novel SXT/R391 ICE was detected, isolated as being associated with a *Proteus mirabilis* strain. Whole genome sequencing using Ilumina sequencing technology revealed an 81 kb element with 75 open reading frames. The "hotspot regions" contained resistance modification systems, toxin-antitoxin systems and a novel BREX (bacteriophages exclusion) system which we are characterising further.

16

Polysaccharide-Dependent Biofilm Formation is induced by Bile in Late Cystic Fibrosis Isolates of *Staphylococcus aureus.*

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Abstract

Cystic Fibrosis (CF) affects 70,000 people worldwide. *Staphylococcus aureus* is the pathogen most frequently isolated from CF patient's airways, particularly children and adolescents. A large percentage suffer from

gastroesophageal reflux disease which can result in bile aspirating into the lungs. A previous study reported bile exposure enhancing biofilm formation of some *S. aureus* strains (Ulluwishewa *et al*., 2016. Microbiology 162:1392-406). Our study investigated virulence factors associated with CF *S. aureus* isolates. Bile induced *S. aureus* biofilms were established *in vitro* using clinically relevant strains and biofilm composition was investigated. Strains CF0, CF11 and CF26 studied were obtained sequentially over 26 months from a CF patient's sputum.While CF11 did not form biofilm under conditions tested, CF0 formed NaCl-dependent biofilm and CF26 only formed biofilm in NaCl-supplemented broth with bile (0.035%) added to the media. *S. aureus* biofilm can be mediated by *icaADBC*-encoded polysaccharide intercellular adhesion (PIA) or surface proteins such as fibronectin binding proteins, protein A and SasG. Proteinase K did not effect biofilm density while sodium metaperiodate degraded CF26 biofilm demonstrating the presence of polysaccharides, allowing us to conclude CF26 forms PIA-dependent biofilms. To examine if bile induced *icaADBC* operon expression, RT-PCR was carried out on RNA extracted. The *icaA* transcript was present at similar levels in bacteria grown in media with and without bile, indicating CF26 failure to form biofilm in the absence of bile is not due to failure inducing the *icaADBC* operon. We hypothesise there may be reduced PIA export or a reduced amount of cell surface anchored PIA.

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Genomic and Proteomic Analysis of the Giant Acinetobacter Bacteriophage vB_AbyM_TRS5

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Abstract

Bacteriophages are viruses which only infect bacteria and are considered the most prevalent biological entities on earth. The increase in antibiotic resistance has reinvigorated research into the use of bacteriophages as therapeutic agents. vB_AbyM_TRS5 (TRS5) is a giant bacteriophage of the family *Myoviridae*, isolated from activated sludge after enrichment with *A. baylyi* ADP1. TRS5 was sequenced using Illumina paired-end sequencing which revealed a genome size of 371.5 kb, the largest known bacteriophage to infect this genus. Giant bacteriophages are rarely isolated but encode many more genes than smaller phages and exhibit more complex virion structure and genome organisation. Here, we report the results of nanopore (minION) sequencing, virion morphology, host range and analysis of virion structural proteins by SDS-PAGE and mass spectrometry. A hybrid genome assembly was performed using Canu and Pilon. These data confirmed the genome size and also identified 672 ORFs and 18 tRNAs. Data collected by ESI-MS/MS identified a total of 66 virion structural proteins, whilst 2D SDS-PAGE identified 54 proteins. Some of these proteins may be delivered into the host cell alongside the virus genome. Host range analysis revealed that TRS5 productively infects strains of *A. baylyi* but also *Acinetobacter baumannii*, identified as a priority 1 critical pathogen for the research and development of new antibiotics. TRS5 is an intriguing candidate for further study, both in terms of phage therapy but also for biotechnology and molecular tools to work with *Acinetobacter sp*..

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D-serine: trick or treat?

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Abstract

D-serine is an amino acid that has become a focus in recent years due to its unique role in many biological processes. It is a host metabolite in humans with diverse roles in neurotransmission and signalling. Previous work in our group showed that D-serine can play a critical role in controlling expression of pathogenic virulence factors in bacteria, specifically *Escherichia coli*, as well as impacting microbial community composition through

niche specificity. In enterohaemorrhagic *E. coli* (EHEC), the presence of D-serine results in down-regulation of the type 3 secretion system (T3SS), resulting in inability to colonise. In contrast, uropathogenic *E. coli* (UPEC) can catabolise this metabolite and colonise the bladder where D-serine is present in high concentrations, leading to a urinary tract infections (UTI). Hence, in different pathotypes, D-serine can act as a positive (treat) or negative (trick) environmental stimulus.
UPEC and neonatal meningitis *Escherichia coli* (NMEC) strains often carry the DsdXCA operon which allows for the metabolism of D-serine. This locus is responsible for the detoxification of D-serine, allowing for tolerance of D-serine as a carbon source. My work has focused on understanding the role of D-serine as a signal for gene expression in UPEC and NMEC through the action of the regulator, DsdC. Using global approaches I have characterised the binding sites of DsdC across the chromosome revealing new insights into how this protein contributes to UPEC and NMEC pathogenesis. The work is important as it helps us understand how specific pathogens sense their environment and cause disease.

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Household arthropods and their associated bacterial communities

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Abstract
Current climate change is affecting arthropod populations all over the world, leading to changes in the number of arthropods and to the establishment of non-indigenous species. Households host a great diversity of life. Beyond microorganisms, arthropods are the most diverse group of organisms found indoors, although knowledge of the diversity of household arthropods excluding pest species is limited. Considering arthropods ability to act as a vector of pathogens, the knowledge of bacteria associated with household arthropods and hence their possible risk to public health is still scarce. The aim of this study is to update knowledge about household arthropods in the UK, exploring their communities in homes located in various areas of Birmingham over a 12-month period and to investigate the microbial communities. In 5 months of collection 537 arthropods were sampled from 17 households. Flies were the most common arthropods collected followed by spiders and beetles. So far 57 pools of different arthropods were analysed and 194 bacterial strains were recovered. The 46.9% of isolates were Gram positive cocci, followed by Gram positive rods (18.6%) and Gram negative rods (16.5%), the remaining percentage is represented by Gram negative coccobacilli, yeasts and filamentous bacteria. The isolates with a high bacterial load were identified to the species level revealing the presence of opportunistic pathogens such as *Staphylococcus* spp., *Acinetobacter* spp. and *Serratia* spp. Upon completion of the study, the resulting information will assist in informing and prioritizing pest control strategies which are under review as a consequence of climate change.

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The Diversity and Comparison of the Temperate Bacteriophages of *Pseudomonas aeruginosa* from the IPCD International Pseudomonas Consortium Database containing over 1000 *Pseudomonas aeruginosa* Genomes.

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Abstract
The accessory genome of *Pseudomonas aeruginosa* (PA), frequently seen as insignificant in comparison to the core genome, typically contains temperate bacteriophage (phage) genomes affecting the bacterial host *P. aeruginosa.* As PA's presence in chronically infected lungs correlates with loss of lung function particularly in cystic fibrosis (CF) and non-CF bronchiectasis (nCFBR) the changes that the temperate phages make can give advantageous effects to their host which may be enable the PA chronically infects. This study gives the wide range look at PA temperate phage, using the IPCD International Pseudomonas Consortium Database

containing over 1000 genomes. These PA genomes were analysed to identify temperate phages within their genomes, the comparison of these phages allowed the diversity of PA phages to be uncovered. The genes of prevalence within the phages from isolates from lung infections were then scrutinised to assess whether they have functions to benefit their PA bacterial host within the lung environment.

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Evaluation of the retention of clinically relevant pathogens on high touch environmental surfaces using the ATP Bioluminescence Monitoring system

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Abstract

Several types of clinically relevant pathogens can persist survival on a range of high-touch inanimate surfaces for example on mobile phones, and visual inspections may be an insufficient measurement to determine their hygienic status. This study evaluated the microbial load retained on high touch surfaces, namely 304 2B finish stainless steel and commonly used tempered glass screen protectors for mobile phones. A 40 mm x 40 mm area of the surfaces was soiled with a 100 µL standardised culture of *Escherichia coli, Staphylococcus aureus* or *Candida* sp., at 10^7 CFU mL^{-1}. The surfaces were cleaned with commercially available antibacterial wipes and the retained microbial load was measured using an ATP Sanitation Bioluminescence Monitoring system (Hygiena Ultra snap, UK).

The stainless steel surfaces were determined to be more hydrophobic (ΔG_{iwi} - 66.6) than the plastic surfaces (ΔG_{iwi} -17.2). The microbial load removed from the surfaces ranged from 55 % - 99.6 % with *Candida* sp. resulting in the lowest removal rates compared to *E. coli* and *S. aureus* (p=0.028). Following bioluminescence monitoring whilst all the stainless steel surfaces achieved the clean status (<30 RLU), 6 % of the tempered glass surface were recorded as requiring further cleaning (>30 RLU). Thus, the more hydrophobic, metal surfaces were deemed more hygienic than the glass surfaces in this study. This work demonstrated that the chemistry and hydrophobicity of the surfaces influenced their surface hygiene.

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Acquisition of fluoroquinolone resistance leads to increased biofilm formation and pathogenicity in *Campylobacter jejuni*.

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Abstract

Aim:

The World Health Organization has listed *C. jejuni* as one of 12 microorganisms on a global priority list for antibiotic resistance due to a rapid increase in the number of strains resistant to fluoroquinolone antibiotics. This fluoroquinolone resistance (FQ^R) is conferred through a single point mutation within the *gyrA* gene which is also involved in DNA supercoiling homeostasis. We recently revealed that changes in DNA topology play a major role in the regulation of virulence in *C. jejuni* with relaxation of DNA supercoiling associated with increased attachment to and invasion of human epithelial cells. The aim of this study was to investigate whether FQ^R of *C. jejuni* resulted in altered supercoiling associated phenotypes.

Method:

A panel of natural mutants were selected against nalidixic acid and ciprofloxacin. Their biofilm formation and virulence were investigated using a combination of molecular and microscopy approaches

Results:

All mutants were shown to have a greater ability to form viable biofilms under aerobic conditions and this phenotype was associated with changes in DNA supercoiling levels. These mutants were also shown to have an

increased ability to attach to and invade epithelial cells *in vitro* and conferred an increase in the killing efficiency of *Galleria mellonella.*

Conclusion:

We report for the first time that fluoroquinolone resistance in *C. jejuni* is associated with an increase in virulence and the ability to form viable biofilms in oxygen rich environments. These altered phenotypes may play a critical role in the continued increase in FQ^R observed for this important pathogen.

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Actinobacterial Diversity from Indonesian Extreme Environments As a Source of Novel Antimicrobial Drug Leads

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Abstract

The search for novel antimicrobial compounds that can be developed to control multidrug resistant (MDR) pathogens is being increasingly found on the isolation and screening of previously unknown actinobacteria from the extremobiosphere on the premise that the condition therein will give rise to population of previously unknown undiscovered actinobacteria which will be the source of new specialized (secondary) metabolites with novel modes of action. Over these past 3 years, two hundred and thirty five isolates of actinobacterial strains in total were successfully recovered from diverse range of extreme habitats in Indonesia. 16S *rRNA* sequencing studies of fifty-two representative isolates has shown that one-third of the isolates identified as putative novel species from *Streptomyces* genus and the rest belongs to the rare genera, such as *Dermacoccus*, *Arthrobacter*, *Pseudonocardia*, *Kocuria*, *Verrucosispora*, *Microbacterium*, *Gordonia*, *Janibacter*, *Kyotococcus*, *Mycobacterium*, *Micromonospora*, *Rhodococcus*, *Nocardioides*, *Micrococcus*, *Actinospica*, and *Amycolatopsis*. Furthermore, standard plug assay also exhibited that 37 out of 52 representative isolates have various inhibition activities against six panel of tested microorganisms. Further investigation using genome based-taxonomy and genomic mining revealed that one of moderately thermophilic strain isolated from arid land habitat, *Streptomyces* sp. PRKS01-29, as a new potential 'goldmine' of antimicrobial compounds. AntiSMASH analysis revealed the presence of 56 biosynthetic gene clusters (BGCs) encode for extremely diverse of secondary metabolites (both known and unknown compounds), such as antibacterial (nigericin, daptomycin, laidlomycin, 7-prenylisatin, phenalinolactone, and teicoplanin), antifungal (ECO-0231, echosides, fengycin, and filipin), anti-virus (nanchangmycin, merochlorin, oxazolomycin, and feglymycine) and antiparasites (macrotetrolide, lasacoid, and aculeximycin).

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Application of ABDITE® support for nitrification for Anammox activity in Expanded Bed Biofilm Reactor (EBBR)

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Abstract

ABDite® is a porous carbon, biomass support material ideal for use in expanded bed biofilm reactors (EBBRs). ABDite® has surface pores that are protected from hydrodynamic shear and, therefore, more easily colonized by bacteria than the exposed surface of solid materials, such as sand. Growth beyond the pores leads to the formation of an enveloping biofilm and, thus, the formation of "bioparticles". The Anammox denitrification process involves several bacteria including Ammonia oxidizing bacteria (AOB) Anammox bacteria and Comammox bacteria, however, the hybrid Anammox process is inhibited by many factors, which hinders the process improvement in continuous culture bioreactors. Anammox process has been established in several

types of fixed film bioreactors, but has not been fully established in EBBRs. Following 60 weeks of continuous culture in a lab scale EBBR (700 cm3) established with ABDite® particles (0.7 – 1.0 mm diameter) and seeded with Anammox granules from an active wastewater treatment plant, the EBBR achieved 44 % maximum nitrogen-removal, at a rate of 1.9 Kg N/m-3d-1. Scanning Electron Microscopy revealed bacterial attachment in the pores of the particles; however, few particles developed complete, enveloping biofilms. Bacterial DNA was successfully extracted from the ABDite® particles using species-specific PCR, Sanger sequencing of products and BLAST analysis, (Nitrosomonas sp. clone A28 and clone M22 (99 – 100 %), Nitrobacter sp. clone BR_02, Nitrobacter sp. FGPS_EFE_3b (75 -99 %), Candidatus Kuenenia, Candidatus Brocadia clone ANA19042 (97 -99 %) and Nitrospira inopinata). We believe this is the first report of Anammox bacteria colonizing ABDite® particles in an EBBR.

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Inactivation efficacy of four model bacteria in plasma activated water during cold storage

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Abstract

In order to investigate the bactericidal effect of plasma activated water (PAW), two Gram-positive bacteria of *Listeria innocua* and *Staphylococcus aureus,* together with two Gram-negative and spoilage bacteria of *Pseudomonas fluorescens and Shewnella putrefaciens* were selected. PAW was obtained by using an atmospheric cold plasma jet (ACPJ) at 15 and 30 kV for 5 min respectively. The planktonic bacteria suspension was mixed with PAW and stored at 4 ℃. During the storage, 0.5 h, 1h and 24 h were selected to evaluate the viable counts. The results showed no inactivation for the two Gram-positive bacteria in 1 h at 15 kV, but *L.innocua* obtained more than 5 $\log_{10}$ CFU/ml reduction after 24 h, while the reduction in *S.aureus* was only around 1.5 unit. At 30 kV, *L.innocua* and *S.aureus* achieved 1.5 and 0.5 unit inactivation respectively in 0.5 h, and no colonies were seen after 24 h. *S. putrefaciens* and *P. fluorescens* obtained 5.5 and 1.6 $\log_{10}$ CFU/ml reduction respectively at 15 kV in 0.5 h, whereas no colonies were seen for the two bacteria at 30 kV in 0.5 h. The study demonstrated that inactivation rate of PAW was in positive correlation to the applied voltage and storage time, and Gram-negative bacteria were significantly more sensitive than the Gram-positive bacteria. The investigation can help to optimize treatment parameters based on target bacteria and also offer the promising possibility of applying PAW as disinfectant in the food industry.

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Staphylococcus aureus targets corneodesmosin to colonise skin in atopic dermatitis

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Abstract

Staphylococcus aureus plays an important role in atopic dermatitis (AD), a chronic inflammatory skin disease. Skin colonization by *S. aureus* exacerbates the clinical severity of AD. However the molecular determinants of adhesive interactions between bacteria and skin are poorly understood. Elucidating the molecular basis of skin colonization is crucial to understand the pathogenesis of AD and to develop new therapeutic strategies. *S. aureus* adheres to dead flattened skin cells known as corneocytes in the outermost layer of the epidermis. Corneocytes in AD skin have an altered surface morphology compared to corneocytes from healthy skin. Corneodesmosin, an adhesive protein normally confined to the tight junctions between corneocytes, decorates the tips of villus-like projections on the surface of AD corneocytes. Here we identify

corneodesmosin as a key ligand for *S. aureus* on AD corneocytes. We show that strain of *S. aureus* isolated from infected AD skin lesions adhere to recombinant corneodesmosin. Using surface plasmon resonance, we show that three cell wall anchored proteins expressed recombinantly bind to corneodesmosin with high affinity. Two of these proteins promote *S. aureus* adherence to corneodesmosin. High-resolution imaging of corneocytes from AD skin revealed that strong adhesive interactions are not uniformly distributed across the corneocyte surface but mostly concentrate on the tips of villus-like projections, consistent with corneodesmosin being a ligand. In summary this study identifies novel interactions between *S. aureus* and corneodesmosin and thus provides important new insights into the first steps in the establishment of *S. aureus* skin colonisation in AD patients.

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Analysis Of Qualitative Feedback Received By Means Of Modified 'Take Five' Antibiotic Audit Tool

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Abstract
Objective: Appropriate antibiotic prescribing is essential for patient care. As a result, we set out to explore reasons behind antibiotic prescribing behaviour and how it might be optimised.
Method: Data were collected over a three month period using an online pre-formed questionnaire, which was disseminated monthly to different departments within our hospital. Our specific analysis focused on the free-text question: 'On reflection, are you aware of any opportunities that could have been taken to improve the quality of this antibiotic prescription'. Thematic analysis was performed to develop relevant codes.
Results: We received 62 free-text responses with suggestions for improvement. The top three categories of response related to: (1) missed opportunity to send appropriate specimen(s) to microbiology; (2) sub-optimal/conflicting documentation; and (3) duration of therapy inappropriately prolonged and/or not specified/justified.
Discussion: The feedback provided is valuable in its capacity to inform decision making at the Trust's Antimicrobial Steering Group, and optimising parts of the system in order to make it easier to do the right thing. Areas for potential improvement, highlighted by feedback received, included: (1) Implementation of reminders on our electronic prescribing system (EPS) to ask if blood cultures have been taken, before commencing antibiotics (2) Providing reminders on our EPS for antibiotic review (3) Documentation of discussions with an infection specialist on our EPS. This project is ongoing, and further data collection and analysis is planned following our implantation of suggested improvements.

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CYANOBACTERIA AS ONE OF THE MOST PROMISING BOTANICAL SUN PROTECTING AGENT; A WAY TOWARDS HEALTHY COSMETICS

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Abstract

Ultra Violet light has adverse effects on skin including mutagenicity and accelerated skin aging. With the depletion of Ozone layer responsible in filtering harmful Ultra Violet rays, humans not only in temperate regions but also in tropical countries are more affected. Thus, use of sunscreens has been more demanded.

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Potential toxicity shown by synthetic Ultra Violet filters in humans directs the attention of consumer on natural Ultra Violet filters. Therefore, aim of this study was to determine Sun Protection Factor of naturally occurring cyanobacteria biomass. Biomass was diluted with alcoholic solutions and absorbance was recorded between 290-320 nm at 5 nm intervals using UV- spectrophotometry. Mansur equation was applied to determine Sun Protection Factor. *Oscillatoriales* sp. showed the highest Sun Protection Factor of 1.57± 0.002345 while *Croococcidiopsis* sp. showed the lowest Sun Protection Factor of 0.05± 0.033216. All the tested strains showed considerable Ultra Violet protection capabilities compared to most of the well-known herbal extracts. Effective sun screen properties along with higher photosynthetic ability, rapid growth, less area and simple nutrient requirement for growth, less capital investment and zero environmental pollution, make these cyanobacteria one of the most promising botanical agents to be used as an effective sunscreen in cosmetic industry.

Keywords: Sun Protection Factor, Synthetic, Natural, Adverse effects

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Whole Genome Sequencing Reveals Genetic Diversity in *Mycobacterium avium* subspecies *paratuberculosis* Population Circulating in Irish Cattle

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Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's Disease (JD), a chronic enteritis, in cattle. Understanding the transmission dynamics of MAP will inform control of JD. Whole genome sequencing (WGS) has been applied to many pathogen systems, where its unprecedented resolution has greatly enhanced our understanding of the molecular epidemiology of pathogen transmission. However, WGS has seen limited application to MAP; here we report the first study of its kind in Ireland.

DNA was extracted from 150 MAP isolates collected from cattle across Ireland. Extracts were prepared into libraries and were sequenced on an Illumina NextSeq500. Sequencing data were processed and analysed using a bioinformatic pipeline.

The phylogeny obtained from sequencing data shows that the MAP population circulating in Irish cattle is diverse, which may partly have resulted from importation of MAP strains from Europe into Ireland. This shows similarity to a WGS MAP study in Canada that noted the impact of cattle imports on the Canadian MAP population. Furthermore, comparing our Irish isolates to a global MAP WGS study showed close similarity of some Irish isolates to European isolates, again suggesting intracontinental spread.

Comparison of WGS data with previously available VNTR data for our isolates indicates that VNTR typing has limited resolution for discriminating MAP strains, and often does not distinguish isolates correctly when compared with WGS SNP data.

The genomic data presented here provide the first comprehensive picture of genetic diversity of Irish MAP and a baseline for future studies into the spread and persistence of MAP in Irish cattle.

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Fibronectin Binding Proteins Mediate Adherence of *Staphylococcus aureus* to the corneocyte protein Loricrin

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Abstract

The bacterium *Staphylococcus aureus* colonizes multiple body sites, primarily the anterior nares, skin and nasopharynx. Colonized individuals are at greater risk of serious *S. aureus* infection. Targeted decolonisation with topical antibiotics is used to prevent infection in specific high-risk groups, such as those undergoing surgery and atopic dermatitis patients, but the efficacy of this approach is threatened by antibiotic resistance. Advances in understanding how *S. aureus* establishes itself on the skin and in the nasal cavity are needed to inform new strategies for the elimination of *S. aureus* carriage in at-risk groups. Loricrin-deficient knock-out mice were previously found to be more resistant to nasal colonization by *S. aureus* than wild-type mice suggesting that the cornified envelope protein loricrin is the main ligand recognised by *S. aureus*.
Here we studied interactions between *S. aureus* CWA proteins and loricrin using purified recombinant proteins and clinical strains. *In vitro* adhesion assays revealed that expression of the CWA fibronectin binding proteins FnBPA and FnBPB in *S. aureus* promoted adherence to loricrin. Null mutations of the *fnbA* and *fnbB* genes in clinical strains reduced their adherence to loricrin. Surface Plasmon Resonance was used to establish the thermodynamic profile of recombinant proteins binding to loricrin and identify the region of the FnBPA and FnBPB proteins containing the binding site. In summary this study identifies a novel interaction between fibronectin binding proteins and loricrin and provides important new insights into the repertoire of *S. aureus* proteins facilitating adherence during colonization.

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Physicochemical Interactions between Silica Nanoparticles and EPS Biomolecules within the Biofilm Matrix of *Pseudomonas fluorescens* WCS365

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Abstract

Difficulties in the removal of bacterial biofilms in the industrial and biomedical sectors have driven the development of new technologies. Although numerous studies have highlighted the use of nanoparticles (NPs) as antibiofilm agents, the fundamental physicochemical interactions between NPs and the biofilm matrix is still poorly understood[1] . The development of “smart nanoparticles” for biofilm removal requires an in-depth understanding of the complex interactions between NPs and biomolecules within the extracellular polymeric substances (EPS) of the biofilm matrix on a nano scale. These interactions are highly dependent on the physical and chemical properties of both the specific biomolecules and the NPs[2]. In order to identify and characterize the specificity of binding and the direct interaction between silica NPs (SiNPs) and EPS matrix components of *Pseudomonas fluorescens* WCS365 biofilms, a range of experiments were carried out. Biofilms were exposed to SiNPs of different charges and surface functionalization, while biomolecules such as proteins, polysaccharides, and eDNA were fluorescently labelled. The distribution, relative abundance and colocalization of SiNPs with EPS biomolecules was quantitatively assessed using CLSM microscopy and advanced image processing. Changes to the SiNPs surface-chemistry dramatically affected their interactions with biomolecules in the biofilm matrix. This includes the increased affinity of SiNPs towards proteins and beta-linked polysaccharides and also lead to changes in the degree to which agglomeration of SiNPs occurs within and on the surface of the biofilm.

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Identification of genes that contribute to fitness of African and global clades of *Salmonella* Enteritidis during infection of macrophages

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Abstract

Non-typhoidal *Salmonella* (NTS) usually cause gastroenteritis in humans, but in recent years NTS have begun to cause epidemics of bloodstream infections in Africa. *Salmonella* Enteritidis is the second most common serovar associated with this invasive form of NTS disease (iNTS) in Africa. To establish a systemic infection, *Salmonella* must be able to survive and replicate within host cells, with macrophages being a primary target. Genomic characterisation of *S*. Enteritidis isolates from human bloodstream has identified two new clades that are unique to Africa and distinct from the global epidemic clade. The African *S*. Enteritidis clades exhibit genomic degradation, and possess a distinct prophage repertoire coupled with multi-drug resistance. However, little is known about the virulence factors that allow African *S*. Enteritidis to cause systemic infection in susceptible hosts. We have found that African *S.* Enteritidis isolates display higher levels of intracellular replication than global *S*. Enteritidis isolates in a murine macrophage infection model, suggesting that there may be clade-specific virulence genes. We therefore screened libraries of random insertion mutants of African and global S. Enteritidis by transposon insertion sequencing (TIS), to identify genes contributing to fitness in murine macrophages. We anticipate our findings will contribute to a greater understanding of African *Salmonella* infection biology, and that some virulence-associated genes in the African clades may be potential targets for developing novel therapeutics in the future.

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Investigating the fundamental interactions between nanoparticles and biofilms of *Pseudomonas* species

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Abstract

The use of engineered nanoparticles (NPs) as a technique for antimicrobial delivery aimed at biofilm treatment is an emerging field of research. Numerous studies involving a wide range of NPs have shown varying results regarding anti-bacterial effects [(1)]. However, research focusing on specific interactions between functionalized nanoparticles and the extracellular polymeric substances (EPS) of biofilms are limited. The complexity of the biofilm matrix may be hindering the understanding of the fundamentals which govern biofilm – nanoparticle interactions.

There are a wide range of physicochemical properties which influence the uptake and retention of nanoparticles within the matrix including NP size and charge properties, biofilm topography and porosity and EPS composition [(2)]. These aspects must be considered when studying biofilm – nanoparticle interactions.

In order to identify these specific interactions, a series of experiments were carried out using mCherry-expressing *Pseudomonas fluorescens* and GFP-expressing *Pseudomonas putida* biofilms. Using high throughput fluorescent intensity measurements and confocal microscopy, it was possible to investigate the uptake of surface functionalized silica NPs by the two biofilms and obtain valuable information regarding biofilm – nanoparticle interactions. The results suggest that specific NP surface functionalization has a major role in guiding the interaction and binding of EPS components, possibly due to electrostatic interactions between NPs and the EPS. The findings of this research will help with the future design of nanoparticles with specific modes of action towards components in the EPS.

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Multimerizing type IV pilus subunit of an oral pathogen binds human cytokines

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Abstract

Aggregatibacter actinomycetemcomitans is a gram-negative biofilm-forming opportunistic pathogen that is associated with inflammatory oral disease, periodontitis. *A. actinomycetemcomitans* binds human cytokines, which may lead to increased virulence. Another gram-negative pathogen, *Neisseria meningitidis*, also binds human cytokines. Its type IV pilus subunits PilE and PilQ have a role in the uptake of IL-8 and TNF-α. *A. actinomycetemcomitans* has similar proteins PilA and HofQ, of which the latter one interacts with cytokines. In this study we focused on the structure of PilA, and its capability to form multimers and to bind human cytokines.

Multimer formation was studied by crosslinking protein multimers with formaldehyde. Results were observed with Western blotting. Three different forms of recombinant PilA were used to determine which parts of the protein are involved in multimer formation. Interaction of monomeric PilA with human cytokines was studied with ELISA.

PilA forms quaternary structures from dimers to large multimers. Its N-terminal α-helix is the most important part in multimer formation. We also showed that PilA binds human cytokines IL-8 and TNF-α. Both cytokines bind to PilA with a micromolar dissociation constants, IL-8 having lower K_d value than TNF-α. The results suggest that *A. actinomycetemcomitans* PilA may have similar features and role in cytokine uptake than *N. meningitidis* PilE.

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Repurposing Old Drugs to do New Tricks – The use of Thioridazine to treat multi-drug resistant infections

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Abstract

Thioridazine (TZ) is a neuroleptic drug with demonstrated antimicrobial activity against a wide variety of microoganisms.The main aim of this study was to understand the potential mechanims(s) of action of TZ, using *Salmonella enterica* serovar Typhimurium as a model bacterium.The antibacterial activity of TZ was determined based on its minimum inhibitory concentration (MIC). Membrane permeability assays were performed *via* the Ethidium Bromide accumulation assay. *Salmonella* was exposed to TZ and its effects on membrane potential and cell wall were assessed by flow cytometry and Transmission Electron Microscopy, respectively. Effects on the bacterial proteome were assessed through 2D gel electrophoresis. Infection assays were performed in THP-1 and RAW 264.7 cells treated and non-treated with TZ.

The MIC of TZ against *Salmonella* was 200mg/L.*Salmonella* treated *in vitro* with sub-MIC exhibited discernible changes in the morphology, only after 15 minutes. TZ disrupts the bacterial membrane leading to leakage of the cellular contents and lysis of *Salmonella*. Proteomic profiling revealed altered expression of proteins involved in permability and efflux. These data demonstrate that TZ mechanim(s) of action mainly involves the bacterial membrane by affecting its permeability and potential. *Salmonella* infected macrophages treated with sub/MIC of TZ showed a decreased on intracellular CFU/mL, suggesting the TZ ability to possibly enhance the killing activity of infected macrophages.

These results suggest that TZ may act *in vitro* by targetting the bacterial cell/envelope. Due to its effect on infected macrophages, TZ may be a useful adjuvant to antibiotic therapy to treat multidrug resistant bacterial infections.

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Unravelling the requirement for host chloride channels during HRSV infection

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Abstract

Ion channels are a diverse class of transmembrane proteins that control ion flux across cellular membranes, influencing a multitude of cellular processes. The modulation of ion channels by viruses is an emerging host-pathogen interaction, and has been demonstrated to regulate critical stages of the virus multiplication cycle including entry, replication, and egress.

Human respiratory syncytial virus (HRSV) causes severe respiratory tract infections (RTIs) globally and is one of the most lethal respiratory pathogens for infants in developing countries, with many cases leading to severe lower respiratory tract infections, and the development of bronchiolitis. Evidence also suggests that childhood HRSV infection contributes towards the increased incidence of adult asthma. There is no HRSV vaccine, and the only treatment is immunoprophylaxis that is prohibitively expensive and only moderately effective; thus new treatment options are required.

We have identified an important role of Cl- channels during HRSV infection, specifically calcium-activated Cl- channels (CaCCs). Time of addition studies using CaCC blockers have allowed us to identify the stages of the HRSV life cycle during which these channels are required. We are now investigating the specific CaCCs facilitating the multiplication of HRSV using genetic means, and well as assessing the importance of Cl- channels in replication cycles of other negative sense RNA viruses.

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Harnessing the *Klebsiella*: macrophage arms race

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Abstract

Klebsiella pneumoniae is a Gram-negative, multi-drug resistant human pathogen causing urinary tract infections, pneumonia and septicaemia. *K. pneumoniae* subverts phagolysosomal maturation and survives in the *Klebsiella* containing vacuole (KCV). In this work, we seek to identify the carbon sources and metabolic pathways used by intracellular *K. pneumoniae*.

In vitro, *K. pneumoniae* is capable of carrying out glycolysis/ gluconeogenesis, the pentose phosphate pathway, the citric acid cycle (TCA) and the glyoxylate pathway. To dissect intracellular *Klebsiella* metabolism, we have followed a genetic approach generating mutants in key enzymes of each pathway. These mutants were assessed for *in vitro* growth kinetics using different carbon sources, adhesion, phagocytosis and intracellular survival in macrophages, and the ability to trigger inflammation. Virulence was assessed by infecting *Galleria mellonella*.

Our results demonstrate that neither bacterial morphology nor growth kinetics in enriched media or minimal media supplemented with glucose are affected by mutations in central carbon metabolism. Loss of the citric acid cycle enzymes malate dehydrogenase and isocitrate dehydrogenase result in poor growth kinetics when supplied only with acetate. Intracellular survival analysis have demonstrated that the enzymes of the glyoxylate pathway are not important for intracellular survival, however interruption of glycolysis/ gluconeogenesis and TCA pathways result in increased macrophage clearance. We have further shown that loss of the enzymes malate synthase and malate dehydrogenase reduce virulence of *K. pneumoniae* in *G. mellonella* infection.

Deciphering the metabolism of *K. pneumoniae* within the KCV may open new avenues of investigation for therapeutic targets to control these infections.

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Macrophage Sabotage: Undermining Macrophage Signalling by *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae has been singled out as an urgent threat to human health due to the increasing number of multidrug resistant isolates. Notably, there are still significant knowledge gaps in our understanding of *Klebsiella*-innate immune system interface. In a previous work of the lab (Ivin et al., 2017), we demonstrated that type I IFN signalling plays an important role in host defence against the infection. In this work, we aim to shed mechanistic light into the role of type I IFN signalling in macrophage intrinsic immunity.

Wild Type and Interferon α/β receptor knockout ($IFNAR^{-/-}$) Immortalised Bone Marrow Derived Macrophages (iBMDMs) cells were infected. Inflammatory read outs were checked along with plating assays and single cell microscopy analysis.

We observed an impaired formation of the phagocytic cup in $IFNAR^{-/-}$ macrophages due to a reduced activation of WASp. Intriguingly, type I IFN signalling is essential for the intracellular survival of *Klebsiella* in macrophages. Lastly, an increase of inflammation was observed in infected $IFNAR^{-/-}$ macrophages suggesting that type I IFN signalling modulates inflammatory responses following *Klebsiella* infection.

Our results show that type I IFN signalling is essential for phagocytosis of pathogens as well as intracellular survival through control of inflammation.

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The putative multicopper oxidase of *Staphylococcus aureus* confers copper tolerance

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Abstract

Staphylococcus aureus is found in the nares of ~20% of the human population persistently as a commensal and is also a leading human pathogen causing a range of infections from minor skin and soft tissue infections to fatal bacteraemia. The success of *S. aureus* can be partially attributed to its ability to resist host immune defences. Copper is a potent bactericidal agent employed by macrophages to enhance bacterial killing inside the phagolysosome. Recent studies have shown that copper hypertolerance genes in *S. aureus* confer resistance to killing in macrophages and whole blood. The contribution of the multicopper oxidase gene (*mco*) of *S. aureus* remains unknown.

The aim of this study was to determine if the *mco* gene promotes copper tolerance in *S. aureus* MRSA252. An isogenic *mco* deletion mutant was created in MRSA252. MRSA252Δ*mco* grew more slowly and to a significantly lower optical density than the wild-type when incubated in broth supplemented with subinhibitory concentrations of copper indicating that *mco* confers copper tolerance to MRSA252. Complementation of the wild-type phenotype was achieved by restoring *mco* at its original locus. MRSA252Δ*mco* was killed more rapidly than the wild-type during incubation in water containing copper, again indicating a copper sensitive phenotype. The contribution of *mco* to the ability of *S. aureus* to resist killing by macrophages was examined by testing the ability of wild-type and Δ*mco* mutant to survive intracellularly. Deciphering the copper tolerance mechanisms of *S. aureus* could lead to therapies that enhance immune killing by attenuating survival of phagocytosed bacteria.

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Secretomic Analysis of Three Ubiquitous *Phytophthora* Species Threatening Global Forest Ecosystems

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Abstract

Phytophthora is a genus of microbial, filamentous eukaryotes that includes some of the most destructive plant pathogens. In this study, we have used mass spectrometry to characterise the secretomes of three understudied *Phytophthora* species that are an increasing threat to global forest ecosystems: *Ph. gonapodyides*, *Ph. chlamydospora* and *Ph. pseudosyringae*. Together, *Ph. gonapodyides* and *Ph. chlamydospora* represent the two most widespread *Phytophthora* species having been found in a wide range of habitats globally. *Ph. pseudosyringae* is a more destructive pathogen and has been identified as having a role in the decline of oak and beech forests across Europe and America. To date, virtually no molecular studies have been performed on these species. Here, we profile the secretomes of these three *Phytophthora* species using an LC-MS/MS strategy. Our approach allowed for the identification of large numbers of proteins secreted into different growth media. We detected a number of important effector families including necrosis-inducing proteins and elicitins, as well as a large number of CAZymes involved in the breakdown of exogenous carbohydrates. Our results provide insights into the molecular mechanisms of *Phytophthora* infection.

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An antibiotics mediated evolutionary arms race between Alexander Fleming's *Penicillium rubens* and a bacterium *Bacillus muralis*.

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Abstract

In the last few decades, considerable attention has been given to study the evolution of antibiotic resistance, but evolution of antibiotics has largely remained understudied. In an antibiotics-mediated evolutionary arms race, the synthesis and release of antibiotics evolves in a group of organisms and their targeted antagonists counter this by evolving antibiotic resistance. It seems plausible that as antibiotic resistance evolves, the antibiotics themselves will evolve too. Therefore, this study used the strain of penicillin producing *Penicillium rubens* discovered by Alexander Fleming to investigate evolutionary modification of penicillin synthesizing gene clusters. The fungus was co-cultured with penicillin susceptible bacteria *Bacillus muralis*. The ultimate aim is to produce novel analogues of penicillin G as a response to evolution of penicillin resistance in the bacterial antagonists of the fungus. Theoretically, due to competition for space and resources, selection pressures would be imposed on the gene clusters harboured in the fungus to produce novel penicillin analogues to continually inhibit the bacterial antagonists.

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The use of genotypic screening, PCR-based replicon typing, pulsed-field gel electrophoresis genotyping and whole genome sequencing to paint a fine transmission map of group 1 CTX-M β-lactamases in ESBL-producing *Escherichia coli* strains isolated from Croatian patients

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Abstract
Aim: This nation-wide, multicentre study aimed to characterize ESBL-producing *Escherichia coli* (*E. coli*) isolates causing hospital-onset, long-term care facility and community urinary tract infections throughout Croatia, and to appraise their genetic resistance markers and plasmid types.

Methods: A total of 164 clinical ESBL *E. coli* isolates have been included. Molecular detection of resistance genes was done by PCR mapping with primers for IS26 and ISEcpI, combined with forward/reverse primers for $bla_{CTX\text{-}M}$ gene. PCR-based replicon typing was used to type the resistance plasmids carrying ESBL genes. Pulsed-field gel electrophoresis genotyping of XbaI-digested genomic DNA was performed with CHEF-DRIII system and Gel-Compar software. Finally, we sequenced a subset of bacterial genomes using the Ion Torrent Personal Genome Machine. The obtained reads were assembled *de novo* using SPAdes 3.11.1; proper assembly was assessed using QUAST, and the contigs were analysed with RAST pipeline and ResFinder.

Results: In addition to bla_{TEM} and $bla_{CTX\text{-}M}$ genes, all except one tested strain harboured *aac(6')Ib-cr* encoding aminoglycoside and fluoroquinolone resistance. The isolates from the Eastern part of the country possessed plasmids predominantly belonging to IncB/O group and IncW group; conversely, the most prevalent plasmid types in central Croatia (the capital) were FIIs and FIA, and FIA was the dominant plasmid type in southern Croatia. All representative isolates belonged to the widespread clone ST131.

Conclusion: This study demonstrated a dissemination of group 1 CTX-M-positive *E. coli* in different geographic regions of Croatia and different arms of the health care system, with striking regional differences regarding plasmid types.

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Role of the exporter PptAB and the protease Eep in secretion and maturation of pheromones in *Streptococcus thermophilu*s

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Abstract
Bacteria use cues from environment to communicate between them-selves. Quorum-sensing (QS), one of the bacterial communication mechanism, let bacteria to behave as a group and to control functions as biofilm formation or virulence. In Gram positive bacteria, auto-inducers or pheromones which are mainly oligopeptides activate QS. *Streptococcus thermophilus* used in dairy industry have QS systems with pheromones called SHP (Short Hydrophobic Peptides) in association with transcriptional regulators of Rgg family.
In *S.thermophilus*, we want to dissect the role of PptAB, an ATP Binding Cassette family transporter, known to export SHP pheromones in *Streptococcus pyogenes* and *Streptococcus mutans* .We also want to determine the role of Eep, a membrane zinc metalloprotease, suggested to be involved in the maturation of some pheromones in *S.thermophilus* .
We used a genetic approach with transcriptional reporters where *luxAB* are controlled by promoter regions of target genes of the pheromones of three QS systems. Only reporters from wild-type (WT) strain emitted light compared to Δ*pptAB* strain. To valid PptAB's role in secretion of SHP pheromones, we researched for mature forms in supernatants of WT and Δ*pptAB* strain by mass spectrometry (MS). To show Eep's role in maturation, we equally used this latter approach with WT and Δ*eep*. Resultantly, mature forms were detected only in supernatant of WT strain.

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Our first results show the involvement of PptAB and Eep in secretion and maturation of three pheromones of *S.thermophilus*. We now want to identify new QS systems involving these two actors with a global transcriptomic analysis.