QRW Abstracts: Pathogen Genomics Meeting

PG1: Genomics for responding to emerging bacterial public health threats

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Public Health Microbiology reference laboratories fulfil a critical role in providing overarching testing and surveillance for notifiable, emerging and foodborne pathogens. These duties require the laboratory to possess an extensive repertoire of validated assays and the ability to rapidly respond to novel threats and outbreaks. For these, among other reasons, the “one stop shop” approach of whole genome sequencing (WGS) has been embraced by public health microbiology laboratories.

Public health genomics played a critical role in Australia’s response to the COVID-19 pandemic and public health laboratories leveraged off existing networks and collaborations to create harmonised national sequence surveillance. This talk will discuss how public health genomics is moving beyond the pandemic and playing an integral real time role in the way disease clusters and outbreaks are not only discovered but in informing on transmission and public health responses and control measures.

Case studies will be discussed focusing on how genomics is being utilised for a number of emerging pathogens causing concerning disease clusters in Australia including *Burkholderia pseudomallei, Corynebacterium diphtheriae* and incursions of multi-drug resistant bacteria of public health importance. The application of genomics for metagenomic approaches to address both diagnostics and surveillance challenges caused by current microbiology testing algorithms will also be considered.

PG2: There's a phage for that… Progress towards identifying promising pathogens and their phages in the primary industries.

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Bacteriophages (phages) are the viruses of bacteria and these parasites appear to be a ubiquitous aspect of cellular life. Phages have received growing attention for their utility as a biocontrol agent that can be used in phage therapy in which pathogens are managed, treated or prevented through the application of phage cocktails (combinations).

While there is much enthusiasm for these approaches, not all pathogens are equally likely to be good candidates for a phage intervention. Herein I will relay what we have learned in this space working on the honey bee pathogen *Paenibacillus larvae*. This bacterial pathogen causes the American Foulbrood disease. In New Zealand, if an apiarists finds signs of this disease, the hive and its occupants must be burned[1].

Using a community science approach, we have discovered, adapted[2] and sequenced 26 novel *P. larvae* phages[3]. We have tested these against a set of 66 *P. larvae* strains from across the country and have found that our phages infect 97% pathogen coverage amongst our phage collection. Assembling effective cocktails and field testing these is ongoing work that will be supported though our new Endeavour Programme which will involve a set of pathogens from the primary industries.

There may be phages for all pathogens, but not all phage hunts will uncover a phage collection with sufficient coverage of host diversity to be effective for therapy. I will speak about what we are learning in this space about selecting pathogens for phage biocontrol and some of the directions we will pursue in the future.

[1] Kok, D. N., & Hendrickson, H. L. (2023). *Save our bees: bacteriophages to protect honey bees against the pathogen causing American foulbrood in New Zealand*. New Zealand Journal of Zoology, 1–16.

[2] Kok, D. N., Turnbull, J., Takeuchi, N., Tsourkas, P. K., & Hendrickson, H. L. (2023). *In Vitro Evolution to Increase the Titers of Difficult Bacteriophages: RAMP-UP Protocol*. PHAGE (New Rochelle, N.Y.), 4(2), 68–81.

[3] Kok, D. N., Zhou, D., Tsourkas, P. K., & Hendrickson, H. L. (2023). *Paenibacillus larvae and their phages; a community science approach to discovery and initial testing of prophylactic phage cocktails against American Foulbrood in New Zealand*. Microbiome Research Reports, 2(4). https://doi.org/10.20517/mrr.2023.16

PG3: A genomics approach to investigate phytoplasma taxonomy and their diversity in Australia.

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Phytoplasmas (family *Acholeplasmataceae,* class *Mollicutes*) are fastidious phytopathogenic bacteria that impact agricultural crops globally. Since they remain to be cultured, metagenome sequencing and genetic analysis offer an opportunity to investigate phytoplasma taxonomy, biology, and epidemiology. In this study phytoplasma genome sequence data of naturally infected samples collected primarily from Australian vegetable growing regions was used to investigate phytoplasma taxonomy and species diversity in Australia. A novel phytoplasma enrichment method based on centrifugation of infected plant material through an iodixanol density gradient was developed that increased the coverage, contiguity, and completeness of the phytoplasma genomes obtained using metagenomic sequencing[1]. A whole genome-based taxonomic delimitation approach was also applied to 11 global 16SrII phytoplasma subgroups or subgroup variants, and one 16SrXXV strain used as an outgroup[2]. It provided several, well-supported lines of evidence to delimit nine species, seven of which were novel species descriptions. The high-resolution genome-based analysis also supported the concept of subspecies within the 16SrII phytoplasma group. Lastly, phytoplasma genomes were obtained by metagenomic sequencing of over 190 samples from Australian vegetable growing regions[3]. The main phytoplasma species and subspecies affecting vegetable crops were determined and novel putative species and subspecies were identified. This research has significantly improved the availability of phytoplasma genomes, contributed to a cohesive taxonomic system for the '*Candidatus* Phytoplasma' provisional genus and demonstrates the importance of accurate species descriptions towards improving our understanding of phytoplasma epidemiology.

[1]Rodrigues Jardim, B., et al (2022) *Iodixanol density gradients as an effective phytoplasma enrichment approach to improve genome sequencing*. Frontiers Microbiology 13:937648.

[2]Rodrigues Jardim, B., et al (2023) *The observation of taxonomic boundaries for the 16SrII and 16SrXXV phytoplasmas using genome-based delimitation*, International Journal of Systematic and Evolutionary Microbiology 73:005977

[3]Rodrigues Jardim, B., et al (2024) *A metagenomic investigation of phytoplasma diversity in Australian vegetable growing regions* Microbial Genomics 10:001213

PG4: Rapid identification and genomic characterisation of Pepino mosaic virus by Oxford Nanopore sequencing, during a New Zealand outbreak

Waite, D.W.[1], Veerakone, S. [1], Delmiglio, C. [1], Kanchiraopally, D. [1], Kelly, M. [1], Khan, S. [1], Liefting, L. [1], Lilly, S.T. [1], Perez-Egusquiza, Z. [1], Tang, J. [1], Yan, J. [1], Tomiczek, L. [2], Thompson, J.R.[1]

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Pepino mosaic virus (*Potexvirus pepini*) (PepMV) is a global threat to tomato production, capable of causing significant economic loss in crops through degradation of fruit quantity, quality, and loss of market value. PepMV is a regulated and notifiable organism in New Zealand. In early 2021, PepMV was detected in a commercial greenhouse[1], triggering a biosecurity response to contain the spread of the virus and limit the severity of infection. Complicating decision-making during the effort was the well-documented highly variable virulence of PepMV, as within strains variants may cause aggressive to mild symptoms. Having knowledge of such information is crucial during an incursion as strategies such as containment or destruction of infected material hinges on the economic and ecological risk that the virus presents.

We applied a previously reported Oxford Nanopore Technologies (ONT) workflow for sequencing plant virus RNA[2] to provide rapid information to decision makers regarding the genotype of the PepMV strain. Within five days of reporting, an ONT library had been prepared and sequenced, confirming infection with the CH2 clade of PepMV and the presence of a closely related potexvirus, potato virus X (PVX). After careful separation of PepMV and PVX sequences, additional analysis of single nucleotide polymorphisms across the genome were used to characterise the strain's potential for virulence. Based on previously characterised markers of pathogenicity in CH2, we classified this isolate as belonging to the mild form of PepMV and were able to inform the course of action of the ongoing response in real time.

1. Veerakone S., Waite D.W., Delmiglio C., *et al.* (2024) *Detection, Characterization, and Distribution of the First Case of Pepino Mosaic Virus in Aotearoa New Zealand*. Plant Diseases. 108(2): 291-295. https://doi.org/10.1094/pdis-02-23-0381-sc

2. Liefting L.W., Waite D.W., Thompson J.R. (2021) *Application of Oxford Nanopore Technology to Plant Virus Detection*. Viruses. 13(8): 1424. https://doi.org/10.3390/v13081424

PG5: Comparative Genomics of ST1 *Staphylococcus aureus* in New Zealand

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Staphylococcus aureus (*S. aureus*) is a bacterium adapted to multiple hosts with an ongoing risk of zoonotic transmission. In 2014, a New Zealand (NZ) molecular epidemiology study revealed that ST1 was the dominant sequence type of *S. aureus* in humans (1). This bacterium is also a significant cause of contagious mastitis in dairy cattle, with ST1 dominating in NZ. This finding is unusual, as international studies report ST97/ST151 as the dominant sequence types in bovine populations. To explore the zoonotic potential of ST1 *S. aureus*, we used a One Health approach and conducted comparative genomics of human and bovine ST1 isolates. This analysis involved Illumina sequencing of 460 bovine isolates from fifteen regions across NZ, and nine human pathology isolates from three regions within NZ. Additionally, we included 183 human and 132 bovine sequences from public databases. As no bovine ST1 reference genome existed, we generated one utilising nanopore and Illumina sequencing. Phylogenomic analysis revealed clustering by host species—however, nine bovine isolates clustered within a predominantly human-associated cluster. Notably, one bovine isolate differed by only 31 SNPs from a closely related human isolate and shared a virulence profile similar to related human isolates. A combination of results led us to believe contamination was the likely cause of the bovine isolate in question. Additionally, our findings indicate that acquiring a well-characterised bovine mobile genetic element may be crucial for host adaptation in bovine *S. aureus* isolates. Our study underscores the need for further NZ-focused One Health research to develop effective strategies for managing and protecting public health and the agricultural sector from high-risk pathogens.

1. Heffernan H, Bakker S, Woodhouse R, Dyet K, Williamson D.A. *Demographics, antimicrobial susceptibility, and molecular epidemiology of Staphylococcus aureus in New Zealand, 2014*. Institute of Environmental Science and Research (2015). (Accessed June 2024).

PG6: The genomics of the New Zealand *Mycoplasma bovis* outbreak

Biggs, P.J.[1,2,3], Binney, B.[4], Foxwell, J.[4], Little, A.[4], Gias, E.[4], Jauregui, R.[4], Sawford, K.[5,6], French, N.P.[1], Burroughs A.[5]

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Mycoplasma bovis (*M. bovis*) is a bacterium that can cause a range of serious conditions in cattle, including mastitis (that doesn't respond to treatment), pneumonia, arthritis, plus late-term abortions. It is described as an Unwanted Organism under the Biosecurity Act 1993. Its discovery in South Canterbury in July 2017 was the first time the disease had been seen in New Zealand.

A multi-faceted surveillance and, later, eradication programme was established by MPI and industry partners, DairyNZ and Beef + Lamb New Zealand around its response to *M. bovis*. One part of this was genomic analyses involving researchers from Massey University, feeding into subsequent genomic epidemiological modelling. Early on, two isolates were chosen for PacBio sequencing to generate reference genomes. Over the last 6.5 years, 1030 isolates associated with the original incursion have been whole-genome sequenced and analysed using nullarbor2 with the two reference genomes. The isolates are all of sequence type 21 with some SNP variants emerging with clonal expansion over time. With input from concurrent epidemiological investigations, the isolates were grouped into fours clades, of which Clade 2 predominated in the outbreak's latter stages. Long read sequencing has now been performed on an exemplar isolate from each clade.

Sequencing alignments were processed to remove recombination – an issue for *M. bovis* – to produce SNP alignments of ~1350 bp for further analysis. Alignment rates were lower than expected due to the large number of insertion sequences within the small ~1Mb genome of *M. bovis*. Pangenome graph methodologies and clade-specific analyses to see if non-linear genome maps can help with mapping rates and allow us to glean more information from this outbreak. Phylogenetic analyses performed during the outbreak have been consistent and have shown the power of genomic epidemiology to inform a response in a live ongoing pathogen outbreak of national significance.

PG7: Genomic data for public health surveillance at ESR.

Winter, D.J.[1], The Health Group[1].

[1]Institute of environmental science and research limited (ESR)

Genomics is a core and growing part of pathogen surveillance at ESR, which cuts across our national reference laboratories, organisms, and reporting lines. Whole genome sequencing is now the primary assay used for public health reporting of enteric bacteria, Legionella, *Streptococcus* spp*, Neisseria meningitidis,* Carbapenemase producing organisms and outbreak investigations. In this talk I will describe how the genomic approaches were validated against existing data to support this transition and describe the public health value gained from genomic surveillance. I will focus on how genomic data supports integrated analysis into source-attribution, transmission tracking and the antimicrobial surveillance in the community.

PG8: A tale of two (and a half) outbreaks: reducing the impact of hospital-acquired infection via prospective genomic surveillance.

Bloomfield, M[1]

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The Wellington Hospital Neonatal Intensive Care Unit (NICU) provides advanced care for extremely premature infants. This population is highly vulnerable to hospital-acquired infection.

Staphylococcus aureus represents an important NICU pathogen, and over the past two decades there have been significant outbreaks of multi-drug resistant *S. aureus* in the Wellington NICU. In 2004-2005 a large outbreak of gentamicin-resistant *S. aureus* (GRSA) occurred. This outbreak was not detected for several months, at which point there had been widespread transmission and several infant deaths. In 2018 an outbreak of methicillin-resistant *S. aureus* (MRSA) occurred, which resulted in two infant deaths and widespread service disruption.

In 2022 our laboratory instituted a prospective genomic surveillance program using Oxford Nanopore sequencing. As part of this, all MRSA isolated from the NICU is sequenced. In 2023 another outbreak of MRSA occurred, however this outbreak was detected by this surveillance system when only two infants were known to be colonised. Due to this early detection the outbreak was contained rapidly, no infants developed significant MRSA infection, and there was little disruption to services.

A key feature differentiating these outbreaks was the availability and timeliness of organism typing, which has changed considerably since the GRSA outbreak. In this talk I will compare the detection and management of these outbreaks and reflect on the significant advances in organism typing that has occurred across these outbreaks, leading to our current system of prospectively detecting in-hospital transmission events.

1. Bloomfield, M., Hutton, S., Velasco, C., et al (2023). *Oxford nanopore next generation sequencing in a front-line clinical microbiology laboratory without on-site bioinformaticians.* Pathology. 56:444-447. doi: 10.1016/j.pathol.2023.07.014

2. White, R., Bakker, S., Burton, M., et al (2024). *Rapid identification and subsequent contextualisation of an outbreak of methicillin-resistant Staphylococcus aureus in a neonatal intensive care unit using nanopore sequencing.* Microbial Genomics. (accepted manuscript)

PG9: Implementation and Optimization of the Oxford Nanopore MinION and its clinically utility at Dunedin Hospital.

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[2] Department of Microbiology and Immunology, University of Otago, Dunedin

Traditionally, outbreaks are detected in hospitals based on the recognition of an epidemiologically linked cluster of cases from which the same organism with the same antibiotic susceptibility pattern has been isolated. Confirmation requires strain typing at a reference laboratory. This approach offers limited sensitivity and specificity to identify inpatient transmission events and outbreaks.

Utilising the Oxford Nanopore MinION, we have implemented whole genome sequencing of bacteria of potential infection prevention control interest in our diagnostic laboratory with the aim to rapidly detect any outbreaks in Dunedin hospital. This has the potential to reduce the morbidity, mortality, and costs associated with hospital acquired infections while preventing unnecessary outbreak investigations when cross-transmission is not proven.

The optimisation and implementation of MinION sequencing in our diagnostic laboratory will be discussed, including the challenges of DNA extraction, and the development of a bioinformatic pipeline using Galaxy that can be run without the ongoing need for specialist bioinformatic support. In the short time since implementation we have already shown clinical benefits for patients and the wider healthcare setting. Application of this technology to an outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in our neonatal intensive care unit, an investigation of a possible cross-transmission event in the adult intensive care unit, and an outbreak of a carbapenemase-producing *Escherichia coli* at a long term care facility will be discussed.

PG10: Integrating onsite whole-genome sequencing with national surveillance and public data for rapid outbreak identification and management

White, R.T.[1], Bakker, S.[1], Balm, M.[2,3], Burton, M.[2], Castro, M.L[1], Clissold, C.[3], Dyet, K.[1], Eustace, A.[1], Hutton, S.[2], Kelly, M.[3], Macartney-Coxson, D.[1], Tarring, C.[2], Velasco, C.[2], Bloomfield, M.[2,3]

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Hospital-acquired infections (HAIs) threaten patient safety. By linking antimicrobial resistance (AMR), single-nucleotide variants (SNVs) analyses, and epidemiology, the Institute of Environmental Science and Research (ESR) provides advice on emerging issues.

In collaboration with Awanui Labs Wellington, we implemented an onsite whole-genome sequencing programme at Wellington Regional Hospital, using Oxford Nanopore Technologies (ONT). Focusing on HAIs, we sequenced cultured isolates from inpatients using the MinION device. Multi-locus sequence typing data were then cross-referenced with patient bed movement data from the hospital's electronic data warehouse. Retrospective phylogenetic analyses used core-genome SNVs and comparisons with global and national genomes.

From February to October 2022, the isolates from 76 out of 118 *Clostridioides difficile* infection (CDI) cases were successfully sequenced. There was substantial sequence type (ST) variation across cases, with only four different STs present in more than four cases. A predominance of cases with ST2 isolates emerged among patients in the hemato-oncology ward between May and October 2022, totalling ten patients. Following targeted interventions, including a change in cleaning products, no further outbreak-associated ST2 cases were detected. We later detected and controlled an outbreak of ST97 methicillin-resistant *Staphylococcus aureus* (MRSA) in the Neonatal Intensive Care Unit (NICU). The outbreak was identified 13 days after the first MRSA-positive culture, initially involving only two known cases. Rapid ward screening identified six additional colonised infants. Effective infection control measures minimised further transmission, with only two additional ST97 cases and three unrelated MRSA cases reported.

These experiences highlight the benefits of prospective genomic surveillance using ONT sequencing in identifying and contextualising HAIs. Rapidly identifying a CDI outbreak in the hemato-oncology ward and an MRSA outbreak in the NICU demonstrates this programme's potential to enhance infection control and improve patient outcomes. However, if individual labs increase their independent efforts without coordination, it may undermine national surveillance capabilities, making collaboration essential.

PG11: Pathogen genomics for public health action

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Genomics has become an essential tool for public health surveillance, action, and strategy, proving its value over the course of the COVID-19 pandemic. These new and evolving technologies have had a remarkable impact on how the public health system monitors and responds to threats. Genomics is important for several critical public health activities including surveillance, testing, contact tracing, tracking genomic changes and adaptations, detecting emerging threats, vaccine selection, and monitoring of outbreaks. Genomic data, from whole genome sequencing to wastewater, provide complementary surveillance tools that, used together, give a 'micro' and 'macro' lens on how a pathogen is behaving in the population. In this talk, we will describe how genomic technologies were used for public health action over the COVID-19 pandemic, and more recently for outbreaks of other pathogens in Aotearoa.

PG12: Challenges and Advances in Understanding *Cryptosporidium* in New Zealand

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Cryptosporidiosis is a neglected infectious disease, caused by single-celled *Cryptosporidium* apicomplexan parasites. *Cryptosporidium* has serious impacts on infants and immunosuppressed. Human infection may be from other people, animals or via the environment (including food and water) and caused by different species and subtypes. Understanding *Cryptosporidium* epidemiology, however, has been difficult because in addition to cases often being exposed to multiple risk factors, the parasite is difficult to sequence.

Most clinical samples do not contain sufficiently large numbers of oocysts to reach the minimum DNA requirements for sequencing library preparation. Oocysts shed in faeces have ~40fg of genomic DNA and a hard oocyst wall. Sequencing from environmental samples is even more challenging. Moreover, cell culture has proven very difficult, and cloning is impossible, so *Cryptosporidium* has mostly been propagated in immunosuppressed animals for research. *Cryptosporidium* undergoes asexual and sexual multiplication and has four related haploid sporozoites within an oocyst, so most genomic data have been generated using populations of parasites. Though a eukaryote, *Cryptosporidium* also has no mitochondrial DNA.

Together, these features create considerable challenges for genomic and epidemiological analyses of *Cryptosporidium*. Most studies use single loci PCR of variable regions, such as gp60, and Sanger sequencing. However, today there are approximately 700 *C. parvum* and 400 for *C. hominis* genomes that have revealed more complex global population structures.

Here, we review the historic and current *Cryptosporidium* situation in New Zealand, including the recent large outbreak in Queenstown, during which gp60 Sanger and metabarcoding sequencing were used to link cases. We will also discuss how different techniques are being used to try to understand *Cryptosporidium*, including the use of single cell manipulation, whole-genome sequencing, transcriptomics and metabarcoding, to help better understand the epidemiology of human cryptosporidiosis in New Zealand.

PG13: Identification of *Staphylococcus aureus* genes that predispose children with osteomyelitis to adverse health outcomes

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Paediatric acute hematogenous osteomyelitis (PAHO) is a bacterial bone infection usually treated successfully with antibiotics following hospitalisation. A subset of patients experience recurrent or chronic infection. Methicillin sensitive *Staphylococcus aureus* (MSSA) is identified as the causative organism in over 80% of cases. The aim of this study was to identify bacterial genes and phenotype in MSSA strains collected from children with PAHO that correlate with complicated or uncomplicated disease.

We performed whole genome sequencing on 94 *S. aureus* isolates, collected from children with well-documented PAHO outcomes treated at Starship from 2008-2017. These included 47 patients with uncomplicated infection (MSSA infection without relapse, <6.5 weeks of antibiotics) and 29 with complicated infection (MSSA infection with chronic/recurrent disease, or treated with ≥8 weeks of antibiotics). Additional isolates from patients with MRSA infection or who were admitted to PICU but did not meet the complicated criteria were sequenced. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays for selected antibiotics were performed.

The isolates were assigned to 33 different sequence types (ST) using the MLST classification, including 9 previously unreported STs. The most common were ST1 and ST5 (mainly uncomplicated). All 6 cases with ST121 were in the complicated or PICU group. Phylogenetic clustering based on the core genome showed clustering of uncomplicated and complicated cases. Presence of antimicrobial resistance genes did not predict clinical outcomes. Likewise, MIC for flucloxacillin and cefazolin was similar for both uncomplicated and complicated groups. GWAS analysis identified the virulence genes *lukS-PV* (a subunit of the PVL toxin), *vWbp* (an immune evasion factor), and in the whole genome *hlgC* and *lukDv* as genes likely to be absent in uncomplicated infection. In conclusion, our study suggests that pathogen genetics influence clinical outcomes in PAHO and that strain genotyping could help to identify patients at risk of more severe infection.

PG14: Exploring collateral vulnerabilities in drug-resistant Mycobacterium tuberculosis: A path to novel anti-TB therapies

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Drug-resistant (DR) *Mycobacterium tuberculosis* (*Mtb*) strains are a severe threat to public health globally. The emergence of drug resistance in pathogens is often linked to mutations in core metabolic pathways. However, such mutations are often at the cost of fitness, resulting in reduced growth, survival and virulence. We hypothesised that specific pathways become more essential for the growth and survival of DR strains to compensate for fitness costs associated with drug resistance. By targeting these pathways, new therapeutic avenues may emerge. This research aims to comprehensively define collateral vulnerabilities in DR *Mtb* strains on a genome-wide scale and identify potential gene targets for the development of innovative anti-tuberculosis (TB) treatments.

We employed Whole Genome CRISPR interference (WG-CRISPRi) to assess gene vulnerability through target inhibition across the entire Mtb genome. By combining WG-CRISPRi screening with transcriptional and metabolomic profiling, we identified collateral vulnerabilities in both an avirulent $mc^2$6206 (H37Rv *ΔpanCD*, *ΔleuCD*) strain, and its INH-resistant mutant (INH^R-*katG*).The WG-CRISPRi screens identified 388 potential gene targets in INH^R-*katG* with increased vulnerability to transcriptional inhibition, ranging from a broad spectrum of biological functions.

This work generated a genome-wide platform for identifying vulnerable genes in an INH^R-*katG* mutant, provided fundamental insights into the mechanisms that allow DR *Mtb* to adapt to the perturbation of DR-conferring mutation, and highlighted how changes in the physiology of drug-resistant strains generate druggable vulnerabilities.

PG15: Developing a non-invasive diagnostic test for Legionnaires' disease using cell-free DNA in urine

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Legionnaires disease (LD), a form of community-acquired pneumonia, is caused by the inhalation of *Legionella* bacteria from a contaminated environmental source. Clinically LD is indistinguishable from pneumonia caused by other respiratory pathogens, and prompt treatment is crucial for patient survival. If LD is suspected, then empiric antibiotic treatment is prescribed prior to diagnosis confirmation. Current diagnostic tests rely on the culture or quantitative polymerase chain reaction (qPCR) of a sputum sample which can be challenging to obtain, as patients are often confused and present with a dry cough.

When microorganisms invade the body, they release fragments of their own DNA that can be found in the blood and urine of patients. Since blood and urine are routinely collected from patients during hospital stays, these samples are ideal for diagnosing infectious diseases. *Legionella* DNA has previously been detected in the blood and urine of infected patients using PCR however sensitivity was poor. Since the levels of microbial cfDNA in blood and urine are low, we have investigated preconcentrating microbial cfDNA from urine using *Legionella* sequence specific probes coupled to qPCR. The beauty of this hybridisation assay is the ability to preconcentrate *Legionella* cfDNA from large volumes of patient urine thereby increasing target DNA while removing background noise from the qPCR.

We have shown that *Legionella* sequence specific probes can isolate *Legionella* DNA from healthy urine samples spiked with known concentrations of DNA. While the hybridisation assay was unable to retrieve 100% of spiked *Legionella* DNA, the amount retrieved was significantly better than a commercial cfDNA extraction kit. This work is proof-of-principle, and future improvements are required such as probe optimisation, wash step automation and increase qPCR sensitivity.

PG16: Insights from genomics of low pathogenicity avian influenza underpin risk assessment and preparedness for high pathogenicity avian influenza in Australia

Wille, M[1,2]

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High pathogenicity avian influenza (HPAI) H5N1 clade 2.3.4.4b is causing a panzootic, with enormous impacts on wild birds and mammals, domestic birds and livestock, and associated human cases. Oceania is the only part of the planet still free from this virus. As part of preparedness activities, key questions around expected route of virus arrival, species susceptibility and roles in disease transmission, expected virus movement within Australia, and likelihood of establishment underpin current risk assessments and preparedness activities. Herein I demonstrate the utility of ecology and genomics studies of low pathogenicity avian influenza (LPAI) to reveal features of recent virus incursion events and features of virus migration across the continent. I will outline the most likely route of virus incursion and some of the surveillance activities focusing on arriving migratory birds. While highly pathogenic avian influenza is not present in Oceania, we will again enter a high risk period for an incursion with the arrival of millions of migratory birds from Asia in the spring, and continued improvements to biosecurity, enhanced surveillance, and other preparation activities should continue to be of high priority.

PG17: AvrRvi4 is a ToxA-like effector of the apple scab fungus, *Venturia inaequalis*, and is recognized by the Rvi4 NLR resistance protein of apple

de la Rosa, S.[1], Winter, D.J.[2], Arshed, S.[3], Bus, V.G.M.[4], Bowen, J.K.[3], Baudin, M.[5], Sannier, M.[5], Dagg, T.J.[1,6], Tarallo, M.[2,6], Mosen, A.M.[1,6], Rocafort, M.[1], Plummer, K.M.[7], Le Cam, B.[5], Schouten, H.J.[8], Bradshaw, R.E.[2,6], Mesarich, C.H.[1,6]

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Scab or black spot, caused by the biotrophic fungus *Venturia inaequalis*, is the most economically important disease of apples worldwide. During infection, *V. inaequalis* resides in the subcuticular environment of apple leaves and fruit, where it secretes an arsenal of virulence factors, termed effector proteins, to promote host colonization and disease. In resistant apple cultivars, however, one or more of these effector proteins can be recognised by cognate resistance proteins to trigger defence responses that ultimately halt *V. inaequalis* growth. In New Zealand, a strain of *V. inaequalis*, NZ203.1, was identified that could overcome the *Rvi4* resistance gene of apple, which encodes a nucleotide-binding/leucine-rich repeat (NLR) resistance protein. To understand how *Rvi4*-mediated resistance is overcome, we set out to identify the corresponding effector gene, *AvrRvi4*, using a bulked segregant analysis based on progeny from a sexual cross between NZ203.1 and a *V. inaequalis* strain that carries a functional copy of *AvrRvi4*, J222. Using this approach, we identified a single gene of interest that encodes a small, secreted, cysteine-rich protein with predicted structural homology to ToxA effectors from other plant-pathogenic fungi. In strain NZ203.1, this gene has been disrupted through the insertion of a transposable element. Notably, the candidate AvrRvi4 protein triggers a weak resistance response upon co-expression with Rvi4 in cells of the model non-host plant, *Nicotiana benthamiana*, indicating that it is AvrRvi4. In support of this, the gene that encodes this protein was found to be independently mutated in several Rvi4-breaking strains of *V. inaequalis* collected from France. Interestingly, *AvrRvi4* belongs to a seven-gene family in *V. inaequalis*, but also has homologs in other plant-pathogenic species of *Venturia*. We are currently investigating whether the proteins encoded by these homologs can be recognized by Rvi4, as this may have implications for cross-species resistance.

PG18: Genomics-informed detection and surveillance to prepare Aotearoa for the existential threat of highly pathogenic avian influenza virus

Waller, S.J.[1], Wierenga, J.[1], de Vries, I.[1], Darnley, J.[1], Taylor, J.[1], Heremia, L.[1], Dubrulle, J.[1], Robinson, Z.[1], McInnes, K.[2], Winter, D.[3], Geoghegan, J.L.[1,3]

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Wild aquatic birds carry a panoply of viruses and act as major vectors of virus distribution at a global scale. Although central to biosecurity, the role that such birds play in the transmission of viruses in Aotearoa, and how this may impact human and animal health in the near future, is unclear. This lack of knowledge, combined with very limited surveillance and resources, leaves Aotearoa entirely unprepared for the introduction of highly pathogenic avian influenza virus and its inevitable impacts on wildlife, agriculture and potentially human health. In this project we sampled sea, shore and water bird populations across New Zealand and its outer islands to better understand viral evolution and transmission as well as identify primary sentinel sites and species to monitor the introduction and spread of novel viral strains. We performed a metatranscriptomic analysis of >1,300 samples from more than 40 species of wild birds. We will uncover the transmission dynamics, evolution and prevalence of avian viruses, particularly influenza virus, harboured by wild aquatic birds to understand their global distribution and the connectivity between migratory and sedentary birds in Aotearoa and its territories including sub-Antarctic and Chatham islands.

PG19: Genomic biosurveillance of the kiwifruit pathogen *Pseudomonas syringae* pv. actinidiae reveals adaptation to selective pressures in New Zealand orchards

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In the late 2000s, a global pandemic of *Pseudomonas syringae* pv. actinidiae (Psa) devastated kiwifruit orchards growing susceptible gold-fleshed cultivars. New Zealand's kiwifruit industry has since recovered, following the successful deployment of new, less susceptible cultivars. However, little is known about the extent to which the Psa population is evolving in orchards since its arrival. As a recently emergent pathogen, Psa provides a unique opportunity to study the role of host selection in disease outbreaks. Over 600 Psa isolates from New Zealand kiwifruit orchards have been sequenced between 2010-2022, from commercial monocultures and a diverse germplasm collection. Interestingly, several Psa isolates from Psa-resistant germplasm vines were found to have effector gene deletions. While effector proteins help pathogens cause disease, they can also be selected against when recognised by a host through effector-triggered immunity. These effector loss isolates partially escape host recognition and thus have increased virulence on resistant germplasm hosts. Comparatively, effector loss is very rare in commercial kiwifruit orchards, where the dominant cultivars lack Psa resistance. However, a few isolates have lost the effector *hopF1c*, mediated by the movement of integrative conjugative elements introducing copper resistance into this population. Following the identification of this variant, *in planta* assays were carried out to determine the pathogenicity and competitive fitness of *hopF1c* loss isolates, to better understand the risk and likelihood of its spread. While there were no differences in individual *in planta* growth, a lab-generated Δ*hopF1c* strain was able to outcompete wild-type on some hosts. Further surveillance was conducted in orchards where these variants were originally isolated, with 6.6% of isolates identified as *hopF1c* loss variants. Ongoing genome biosurveillance of New Zealand's Psa population is recommended to enable early detection and management of variants of interest, particularly as new cultivars are deployed in the coming years.

PG20: Nanopore Long-read Sequencing for Monitoring Plasmids Conferring Carbapenem Resistance in Aotearoa New Zealand

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Antimicrobial resistance (AMR) is a major global health issue. Plasmids carrying AMR genes are particularly concerning because they can spread AMR rapidly, both within and between bacterial species. Using short-read next-generation sequencing (NGS) for AMR surveillance usually results in fragmented plasmid assemblies, thereby restricting our understanding of which plasmids contain AMR genes and how they contribute to AMR transmission. Carbapenemase-producing organisms (CPOs) significantly challenge public health by undermining the effectiveness of last-resort antibiotics against multi-resistant Gram-negative infections. To overcome the shortcomings of NGS and support national surveillance, we used nanopore long-read sequencing (LRS).

To the end of April 2024, approximately 1320 clinical isolates suspected of containing a carbapenemase gene have been referred to ESR for national surveillance, and all of which have been NGS sequenced. Of these, 332 CPOs were chosen for LRS based on diverse species, multilocus sequence type, AMR profiles and plasmid signatures. These 332 isolates exhibited 67 unique carbapenemase genotypes based on NGS across 13 species including Enterobacterales, *Pseudomonas aeruginosa*, and members of the *Acinetobacter calcoaceticus*/*baumannii* complex. Nanopore sequencing revealed 1,200 plasmid contigs, identifying 74 single Inc and 125 multi-Inc type fusion plasmids, with 167 unassigned ones.

About 78% (224/288) of the carbapenem resistance genes were located on plasmids, underscoring plasmids' pivotal role in disseminating carbapenem resistance. Our data highlight the diversity of plasmid signatures and AMR profiles among CPOs, suggesting that LRS could offer deeper insights into AMR transmission. By integrating available internationally data, we analysed the global multilayered spread of carbapenem resistance. We also provided evidence that plasmid transfer contributed to the origins of New Zealand outbreaks, such *as E. coli* ST372 *bla*OXA-181 and *E. coli* ST131 *bla*OXA-48.

Our findings can inform infection control strategies and public health decisions. We have obtained a comprehensive reference dataset for further public health surveillance.

PG21: Harnessing Pathogen Genomics to Combat Antimicrobial Resistance in Fiji and the Pacific

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Antimicrobial resistance (AMR) is a growing threat to global health, with particularly severe impacts in low-resource settings. Of particular concern are carbapenem-resistant *Acinetobacter baumannii* (CR*Ab*), *Pseudomonas aeruginosa* (CR*Pa*), *Escherichia coli* (CR*Ec*) and *Klebsiella pneumoniae* (CR*Kp*). In the Pacific, information regarding the spread of these critical pathogens has been scarce.

In this talk, I will discuss how pathogen genomics was harnessed to address AMR in the Pacific region. Our research has utilized molecular and clinical epidemiology to uncover multiple previously unrecognized prolonged nosocomial outbreaks of CR*Ab* sequence type 2 (ST2), CR*Pa* ST773, and CR*Ec*ST410 , and CR*Kp* ST16 in Fiji's healthcare settings. We also identified CR*Ab* isolates from hospital environmental sources that are genetically linked to clinical strains, highlighting potential sources of infection. Observed high mortality rates associated with these resistant strains, coupled with inappropriate use of carbapenem antimicrobials, further underscore the severity of the issue in Fiji.

Additionally, our findings reveal the transnational spread of CR*Ab* ST2 strains across Oceania, including Samoa, New Zealand, Australia, and India. This emphasizes the urgent need for effective screening, genomic surveillance, and comprehensive AMR monitoring systems throughout Pacific Island countries.

Our work demonstrates the critical need for coordinated AMR surveillance and enhanced genomic capabilities in the region. We call for a need to develop actionable strategies by integrating pathogen genomics with clinical and epidemiological data to improve infection control and antimicrobial stewardship practices, ultimately reducing the impact of AMR on public health in the Pacific.

PG22: Genomics across One Health– winners and cautionary tales

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Genomics has expanded exponentially in the last decade enabled by high-throughput sequencing (HTS) technologies. This has supported a range of scientific questions and disciplines to further the understanding into disease ecology, environmental systems, identify previously uncharacterised microorganisms, and to track pathogens during disease outbreaks. Notably, these studies have played a major role in ensuring vaccines and surveillance systems are suitable for circulating pathogens, and for investigating the microbiome of undiagnosed syndromic cases across One Health.

This presentation will highlight the 'winners' in applying meta-transcriptomics for arbovirus surveillance, metabarcoding for insect host blood meals and identifying a bacterial genomics transmission chain across One Health. The 'cautionary tales' highlight the requirement for high quality, controlled, robust bioinformatic analysis and awareness of reportable detections. An example presented of a 'winner' will be The Australian Biosecurity Genomic Database, a new resource for high-throughput sequencing analysis based on the National Australian Notifiable Disease List of Terrestrial Animals.

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PG23: Comprehensive infectome analysis of an emerging disease in the critically endangered Kākāpō using total RNA sequencing

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When a new disease emerges, it is crucial to determine the cause(s) in order to prevent further spread and develop treatments. However, determining the causative pathogen (if one exists), can be very challenging. Total RNA sequencing can identify the entire 'infectome'—viruses, bacteria, fungi, and eukaryotic parasites, as well as the resistome and host immune responses—making it an ideal tool for pathogen investigation, particularly in endangered host species where challenge studies are not possible.

The kākāpō (*Strigops habroptilus*), a critically endangered flightless parrot endemic to Aotearoa New Zealand, has faced severe population declines and extreme bottlenecks, making it highly vulnerable to disease outbreaks. In 2002, exudative cloacitis, a debilitating disease causing inflammation and ulceration of the vent margin or cloaca, emerged in kākāpō. This disease reduces fertility and often requires extensive antibiotic treatment. The increasing case numbers and widening geographic spread of this disease poses a growing threat to kākāpō conservation. Despite emerging over 20 years ago, the causes of exudative cloacitis remain unknown, with previous diagnostic and environmental investigations yielding no definitive results. Herein, we characterize the infectome of lesions and cloacal swabs from nine kākāpō affected by exudative cloacitis and compare this to cloacal swabs from 45 non-diseased kākāpō to identify potential pathogens involved in this disease. We found no host-infecting viruses, indicating either the disease is not caused by a virus, the virus is too divergent to be detected, or the virus is cleared very quickly. We found three species of bacteria that appear to be associated with the disease, including specific strains found only in the diseased birds. We discuss these findings and their implications.

PG24: Soil microbial communities as indicators of soil condition and land management strategies

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Large scale investigations of microbial genomics can uncover important ecological patterns and relationships, with potential applied outcomes. For example, the inclusion of microbial information when monitoring soil quality is crucial if we wish to gain a better, more accurate understanding of how land management impacts the soil ecosystem. This information can then be applied to preserve our biological heritage as well as the productivity and sustainability of our agricultural industries. However, to achieve this, we need to better understand how microbial communities are impacted by different land uses, and land management strategies. Therefore, we combined large scale amplicon datasets collected at over 600 natural and productive sites throughout Aotearoa New Zealand with targeted 'omics data from two contrasting agricultural systems (pasture [n = 22] and vineyards [n=19]) that utilise a range of management practices, from conventional to regenerative. These data are used to show how microbial community composition and function change under different land uses and specific land management strategies, indicating they can provide biologically relevant insights on the impacts of land use on soil ecosystems. Further, by applying machine learning methods we demonstrate the potential of bacterial communities to indicate key soil characteristics which can be used to determine the condition of the soil. With global estimates that over a third of soil is in a state of degradation, increased monitoring coupled with better land management is crucial to ensure the sustainability of agricultural and pastoral industries. Identifying optimal biological indicators of soil health will be an important monitoring tool to help protect our current and future agricultural industries.

Summary of Abstracts for the Poster Session - Pathogen Genomics

No.	Title	Presenter	Institutions
PG25	Viromes of Antarctic fish resembles the diversity found at lower latitudes	Rebecca Grimwood	University Of Otago, New Zealand
PG26	Revealing the virome of the New Zealand sea lion	Nicola Holdsworth	University Of Otago, New Zealand
PG27	Four years of SARS-CoV-2 genomics in New Zealand	Eszter Scarlett-Herbert	University Of Otago, New Zealand
PG28	Virome Seasonal dynamics of the New Zealand little neck clam, Austrovenus stutchburyi	Isa de Vries	University Of Otago, New Zealand
PG29	Uncovering Virus Diversity in Highly Urbanised Waterfowl	Lia Heremia	University Of Otago, New Zealand
PG30	Spatial variation in the microbiome of the sandhopper Bellochestia quoyana endemic to New Zealand	Jeremy Dubrulle	University Of Otago, New Zealand
PG31	Integrating Genomics and Surveillance: Investigating a Staphylococcus aureus Outbreak in a Neonatal Intensive Care Unit in Wellington, New Zealand	Rhys White	ESR
PG32	Understanding how PBT2-Mediated Manganese Depletion Plays a Key Role in MRSA Antibiotic Resensitisation	Caitlin Cleary	University Of Otago, New Zealand
PG33	A metagenomic investigation into Apteryx rowi dermatosis identifies multiple novel viruses and a highly abundant nematode	Jordan Taylor	University Of Otago, New Zealand
PG34	Genomics-informed diagnostics for infectious disease investigations in the endangered hoiho	Janelle Wierenga	University Of Otago, New Zealand
PG35	Phage-Based Strategies for Managing Listeria monocytogenes in Salmon Products	Jan Haviernik	University of Canterbury, New Zealand
PG36	Environmental monitoring for avian influenza viruses in Aotearoa New Zealand	Allison Miller	University Of Otago, New Zealand
PG37	Investigating the impact of fisheries on endangered hoiho diet, microbiome, and disease susceptibility	Eiren Sweetman	University Of Otago, New Zealand
PG38	Antibiotic resistance in Australian leafy greens pre-farm gate	Sabina Shrestha	La Trobe University/Agribio (DEECA), Australia
PG39	Sequential breakdown of the Cf-9 leaf mould resistance locus in tomato by Fulvia fulva	Carl Mesarich	Massey University, New Zealand
PG40	Loss of aminoarabinose lipid modification leads to cephalosporin antibiotic resistance in Pseudomonas aeruginosa	Maddie Hardie Boys	University Of Otago, New Zealand
PG41	Genetic diversity of Mycobacterium tuberculosis isolates circulating in Māori Communities	Callum August	University Of Otago, New Zealand

PG42	Discovery and directed evolution of primordial chloramphenicol resistance genes from the soil metagenome	Jennifer Francis	Victoria University of Wellington, New Zealand
PG43	Comparison of nasal bacteria to middle ear bacteria in preschool children with chronic otitis media with effusion	Rebecca Walker	University Of Auckland, New Zealand

PG25: Viromes of Antarctic fish resembles the diversity found at lower latitudes

Grimwood, R.M.[1], Waller, S.J.[1], Wierenga, J.R.[1], Lim, L.[2], Dubrulle, J.[1], Holmes, E.C.[2], Geoghegan, J.L.[1,3]

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Antarctica harbours some of the most isolated and extreme environments on Earth, concealing a largely unexplored and unique component of the global animal virosphere. The continent's shift southward and cooling temperatures over 20 million years ago led to a reduction in biodiversity and subsequent radiation of some marine fauna, such as the notothenioid fishes. To understand the diversity and evolutionary histories of viruses in these polar species we used metatranscriptomics to determine the viromes of 11 Antarctic fish species with 248 samples collected from the Ross Sea region spanning the orders Perciformes, Gadiformes, and Scorpaeniformes. Despite decreased host species richness in polar regions, we revealed a surprisingly complex virome diversity in Ross Sea fish represented by 42 novel viruses including orthomyxoviruses, filoviruses, and coronaviruses. Overall, the types and numbers of viruses per host species and individuals sampled were comparable to those of fish in warmer marine environments with higher host community diversity. We also observed a larger number of closely related viruses, likely representing instances of recent and historic host-switching events, in radiated Perciformes (notothenioids) than in the Gadiformes, suggesting that rapid speciation events within this order generated closely related host species with few genetic barriers to cross-species transmission. Additionally, we identified a novel genomic variation in an arenavirus from two *Trematomus* hosts with a split nucleoprotein sequence containing a stable helical structure, indicating potential adaptation of viral proteins to extreme temperatures. These findings enhance our understanding of virus evolution and virus-host interactions in response to environmental shifts, especially in less diverse ecosystems more vulnerable to the impacts of anthropogenic and climate changes.

PG26: Revealing the virome of the New Zealand sea lion

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The New Zealand sea lion/ pakake (*Phocarctos hookeri*) is the largest and only endemic seal in New Zealand. It is classified as endangered due to its low population numbers and limited geographic distribution. Exploring the virome of healthy New Zealand sea lions can accelerate pathogen identification during a potential future disease outbreak. Despite this, we currently know nothing about the viruses infecting this species. We performed a metatranscriptomic virological survey of the New Zealand sea lion using faeces collected from healthy sea lions located on beaches around Dunedin, Otago. Samples included faeces from males and females, across all age groups including juveniles, sub-adults and adults, as well as males born in the subantarctic islands and Otago. Our research has identified novel viral species infecting sea lions spanning at least four viral families including the *Astroviridae*, *Picornaviridae*, *Sedoreoviridae*, and *Spinareoviridae*. Additionally, we detected a wide diversity of dietary-related viruses – particularly those infecting fish that were consumed by sea lions. By revealing the entire virome diversity, richness, and abundance of sea lions across these different groups, we have begun to expand our knowledge on how the ecological factors of hosts shape this composition. This project represents a foundational step for future research with significant applications in sea lion disease monitoring and conservation. It is the first virological survey ever conducted on the New Zealand sea lion.

PG27: Four years of SARS-CoV-2 genomics in New Zealand

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From 2020 to 2024, New Zealand has used genomics to track and trace the dynamics of SARS-CoV-2. Over the four years of the COVID-19 pandemic so far, New Zealand's public health response has been characterised by both strict public health interventions and the subsequent relaxation of these measures. Here, we utilise SARS-CoV-2 genomes to better understand the genomic epidemiology of SARS-CoV-2 during this a period. Analysis of high coverage genomic sequences identified distinct monophyletic clades of New Zealand viral genomes against subsampled international sequences. We review the early dominance by single variants such as Delta and BA.1 and then the shift to co-circulation of multiple Omicron subvariants including JN.1.4, XBB and XBC.

Investigation into the impact of public health policies on transmission dynamics shows that initial responses, including strict border controls, were crucial in controlling the virus's early spread and minimising morbidity and mortality. However, the shift from an elimination strategy to a suppression and mitigation approach was associated with the diversification of the virus into various variants of concern (VOCs) such as Delta and Omicron. We find a correlation with the prevalence of multiple Omicron variants and increased viral introductions. Additionally, changes in the effective reproductive number over time in relation to public policy adjustments, vaccination rates, and mobility patterns, is still undergoing analysis.

PG28: Virome seasonal dynamics of the New Zealand little neck clam, *Austrovenus stutchburyi*

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There is a biased understanding of virus diversity and host-virus interactions due to the nature of virus discovery, with a focus on model-organisms over cross-sectional timelines. Investigating virome dynamics in the New Zealand endemic cockle *Austrovenus stutchburyi* across time and space will bridge this knowledge gap. This little neck clam is of economic, cultural, and ecological importance to New Zealand, but no knowledge has been obtained on the viruses that infect this species. *A. stutchburyi* were sampled monthly and key water parameters were measured across coastal Otago over a one-year timeline. Total RNA was extracted from cockle tissues subject to bulk RNA sequencing. The RNA sequencing data is currently undergoing bioinformatic analysis to identify the entirety of the bivalve virome, how it varies seasonally across time, and what key environmental parameters mediate these variations. Thus far, high abundances of viruses were detected across time and space with most novel viruses unclassified at a taxonomic level reflecting the vast under-sampling of New Zealand species. High viral richness has also been identified with viruses falling across 30 different taxonomic orders. It is expected that these viral communities will vary in correspondence to environmental fluctuations. It is crucial to understand such virome-host- environmental dynamics to establish baseline health knowledge to allow for rapid mitigation strategies in case of any future disease emergence events.

PG29: Uncovering virus diversity in highly urbanised waterfowl

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Avian species such as waterfowl serve as crucial reservoirs for viral pathogens. Throughout history, humans have maintained a close relationship with birds, which has intensified in recent times due to factors such as agriculture, deforestation, and globalisation. These factors, coupled with increased human mobility, heighten the risk of viral spillover events and potential pandemics. Despite this, information regarding viruses in waterfowl in Aotearoa is limited.

This study aims to survey and collect faecal samples from waterfowl in botanical gardens and city parks across Aotearoa, where interactions with humans are frequent. Using a meta- transcriptomic approach, we will analyse these samples to assess the extent of human exposure to viruses carried by New Zealand's urban waterfowl. Our objective is to identify any viruses that may pose a potential risk for future disease outbreaks in humans and other animals. This research will not only provide valuable insights into the viral diversity among New Zealand's urban waterfowl but also strengthen the country's infectious disease surveillance efforts.

PG30: Spatial variation in the microbiome of the sandhopper Bellochestia quoyana endemic to New Zealand

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Invertebrate microbiomes have been the focus of increasing research owing to sequencing platforms and downstream computational analysis enhancements. However, marine crustacean microbiomes are still sparsely characterised. The sandhopper Bellorchestia quoyana is the most abundant amphipod of New Zealand's shorelines and plays vital roles in decomposing and recirculating organic matters. Populations of B. Quoyana are physically isolated from each other and they show clear genetic structuring, providing a setting to study spatial variation in their microbiomes. It is well-established that populations distant from one another share less taxa in common than populations geographically close. Because marine crustaceans are evolving in an environment changing rapidly at local scales (e.i currents, tides, storm, etc.) their microbiome must reflect these fluctuations. Using RNA-sequencing, three pools of twenty sandhoppers' gut microbiomes were compared across three coastal sites in Otago, New Zealand. Microbial abundance and patterns of shared taxa were identified. Species richness was heterogeneous for bacteria, fungi and protists across sites, though at different degrees of heterogeneity. Shared taxa, however, displayed disparities in relative abundance across sites. The archaeal diversity overall was limited while we found a high abundance of bacterial and fungal species found in saltwater, freshwater and linked to human activities. Three new viral species likely to infect sandhoppers belonging to the Orthomyxoviridae, Nyamiviridae and Solinviridae families were also identified. Overall, we showed that New Zealand sandhoppers harbour a rich microbiome, with a mix of terrestrial, marine, and human-related organisms. This study contributes empirically to the discovery and characterisation of microbial diversity in a keystone species residing at the interface between land and sea, enhancing our understanding of the composition and functional roles of microbes in natural ecosystems.

PG31: Rapid identification and subsequent contextualisation of an outbreak of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit using nanopore sequencing

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Outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA) are well described in the neonatal intensive care unit (NICU) setting. Genomics has revolutionised the investigation of such outbreaks; however, to date, this has largely been completed retrospectively and has typically relied on short-read platforms. In 2022, our laboratory established a prospective genomic surveillance system using Oxford Nanopore Technologies sequencing for rapid outbreak detection. Herein, using this system, we describe the detection and control of an outbreak of sequence-type (ST)97 MRSA in our NICU. The outbreak was identified 13 days after the first MRSA-positive culture and at a point where there were only two known cases. Ward screening rapidly defined the extent of the outbreak, with six other infants found to be colonised. There was minimal transmission once the outbreak had been detected and appropriate infection control measures had been instituted; only two further ST97 cases were detected, along with three unrelated non-ST97 MRSA cases. To contextualise the outbreak, core-genome single-nucleotide variants (SNVs) were identified for phylogenetic analysis after *de novo* assembly of nanopore data. Comparisons with global (n = 45) and national surveillance (n = 35) ST97 genomes revealed the stepwise evolution of methicillin resistance within this ST97 subset. A distinct cluster comprising nine of the ten ST97-IVa genomes from the NICU was identified, with strains from 2020–2022 national surveillance serving as outgroups to this cluster. One ST97-IVa genome presumed to be part of the outbreak formed an outgroup and was retrospectively excluded. A second phylogeny was created using Illumina sequencing, which considerably reduced the branch lengths of the NICU isolates on the phylogenetic tree. However, the overall tree topology and conclusions were unchanged, except for the NICU outbreak cluster, where differences in branch lengths were observed. This analysis demonstrated the ability of a nanopore-only prospective genomic surveillance system to rapidly identify and contextualise an outbreak of MRSA in a NICU.

PG32: Understanding how PBT2-Mediated Manganese Depletion Plays a Key Role in MRSA Antibiotic Resensitisation

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Multidrug-resistant bacterial infections, particularly those caused by Methicillin-resistant *Staphylococcus aureus* (MRSA), pose a significant public health challenge, necessitating urgent development of alternative treatment strategies. This study investigates the antimicrobial properties of PBT2, a repurposed zinc ionophore, against MRSA. Our findings reveal that PBT2 disrupts key genetic and metabolic pathways in MRSA through dysregulation of intracellular metal ion homeostasis, leading to antibiotic resensitisation. This effect can be mitigated by external manganese (Mn), suggesting its crucial role in the process.

Using the Nebraska Transposon Mutagenesis Library, we identified four genes—*hemB, gmpI, bioY*, and *pdhA*—involved in the cellular response to Mn depletion in the presence of PBT2 and zinc. We hypothesise that these genes play a crucial role in Mn-mediated protection against PBT2 in MRSA. We generated markerless gene deletions in MRSA USA300 isolate TCH1516 to investigate this hypothesis further. This approach allowed us to determine the specific roles of these genes in metal ion homeostasis disruption and impaired Mn protection.

The insights gained from understanding PBT2-induced genetic and metabolic disruptions provide a promising avenue for combating MRSA infections. Furthermore, this research offers a new perspective on reversing antibiotic resistance in bacterial pathogens, potentially leading to novel therapeutic strategies against multidrug-resistant bacteria.

PG33: A metagenomic investigation into *Apteryx rowi* dermatosis identifies multiple novel viruses and a highly abundant nematode

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Sporadic cases of dermatosis have been reported in wild Ōkārito rowi (*Apteryx* rowi), a species of brown kiwi, for over a decade. The disease exhibits distinctive features, including lesions, lichenification and feather loss. Swab samples and full thickness skin biopsies were collected during a survey of affected kiwi in 2023 for a metatranscriptome-based, total infectome investigation to identify any possible microbial agents associated with the disease. Our approach identified divergent, novel viruses as well as a species of nematode in high relative abundance. Of note, we found a highly abundant hepacivirus within the *Flaviviridae*, but only in some mild cases of dermatitis across all sample types, and in both active and chronic infections. In addition, we found a significant shift in the taxonomic composition of the non-viral microbiome within severe chronic dermatitis cases, and this was largely reflected with an increased abundance of transcripts from a parasitic nematode of the *Eucoleus* genus. While determining the primary cause of disease in critically endangered wildlife like the rowi remains challenging, our detection of novel and highly abundant microorganisms opens new lines of enquiry to investigate their potential association with dermatosis in this nationally iconic species.

PG34: Genomics-informed diagnostics for infectious disease investigations in the endangered hoiho

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Metagenomics can provide a rapid, genomics-informed diagnosis for wildlife disease investigations and identify likely candidates that require further scrutiny. Using a meta-transcriptomic approach, we aimed to investigate the presence of bacteria, fungi, protozoa and viruses to identify possible pathogens responsible for disease impacting yellow-eyed penguins (hoiho). Hoiho are predicted to become extinct on mainland Aotearoa in the next few decades, with disease being a significant contributor to their decline. Diphtheritic stomatitis (DS) has resulted in significant mortality and has recently been identified in over 75% of all monitored chicks. In addition, a new disease termed respiratory distress syndrome (RDS) causing lung pathology was identified in very young chicks with a high mortality, however no causative pathogens were identified.

First, we discovered a novel and abundant gyrovirus in tissue samples from chicks with RDS, sharing only 40% amino acid identity within other gyroviruses discovered in tissue samples from diseased birds. Due to its high abundance and absence of other pathogenic organisms, it is likely that this novel gyrovirus is associated with RDS. Secondly, in oral and cloacal samples taken from chicks with DS, we identified a novel and highly abundant picornavirus, most closely related to other penguin viruses. On evaluation of pre-symptomatic, symptomatic and antibiotic-treated penguins, we found no other obvious pathogens associated with the disease, although variable bacterial abundances were identified that could contribute to opportunistic secondary bacterial infections. Overall, we show that metagenomics is a powerful tool to better understand the potential causative agents of infectious disease.

PG35: Phage-Based Strategies for Managing *Listeria monocytogenes* in Salmon Products

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Listeria monocytogenes is a significant foodborne pathogen responsible for listeriosis, a severe infection particularly dangerous for the elderly, immunocompromised individuals, pregnant women, and newborns. This bacterium can contaminate a wide range of food products, including raw meat, dairy, vegetables, fruits, and seafood, posing a substantial public health risk[1]. Our research focuses on salmon products from New Zealand, which have tested positive for *L. monocytogenes* through routine screening. The primary objective of this study is to isolate *L. monocytogenes* from these salmon products, characterize the isolates using sequencing techniques, and determine whether the contamination arises from a single strain, multiple closely related strains, or diverse unrelated strains.

Once we have established the strain diversity in our target industry, we will be exploring the use of bacteriophages as a targeted solution for managing *L. monocytogenes*. Initially, commercial phage solutions will be tested for their efficacy against the isolated strains. Subsequently, we aim to identify and utilize bacteriophages sourced from New Zealand through a comprehensive phage hunt. The goal is to develop a phage-based solution capable of effectively eliminating *L. monocytogenes* associated with salmon products. The anticipated outcome of this research includes the establishment of a reliable method for managing *L. monocytogenes* using either commercially available phages or newly discovered phages from New Zealand. Additionally, we plan to implement this solution within factory environments and develop a commercial product derived from New Zealand phages for ongoing *L. monocytogenes* management.

This innovative approach has the potential to significantly enhance food safety in the salmon industry, providing a robust method for controlling *L. monocytogenes* contamination and reducing the risk of listeriosis outbreaks.

1. Matereke, L. T., & Okoh, A. I. (2020). *Listeria monocytogenes Virulence, Antimicrobial Resistance and Environmental Persistence: A Review*. Pathogens (Basel, Switzerland), 9(7), 528.

PG36: Environmental monitoring for avian influenza viruses in Aotearoa New Zealand

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Highly pathogenic avian influenza (HPAI) is an emerging concern for governments and people worldwide. Since 2021, a new strain of HPAI has emerged allowing the virus to expand both its host and geographical range[1]. HPAI has killed millions of birds and other wildlife around the world[2] and its recent jump and spread among cattle is a major cause for concern in the agriculture industry[3]. HPAI has not yet been recorded in Aotearoa[4]; however, with the growing threat of its intercontinental spread, including to mainland Antarctica[5], we need to improve our active surveillance. Environmental sampling technologies offer ways to do this without the need for handling animals[6]. We will review and test established environmental sampling methods, including longitudinal *in situ* sampling of various environmental samples, to determine an appropriate method for the surveillance of avian influenza viruses across Aotearoa. This information will be vital for the management of newly emerged subtypes of HPAI, acting as an early warning system to spot the virus when it arrives.

1. Wille, M. and Barr, I.G., 2022. *Resurgence of avian influenza virus*. Science, *376*(6592), pp.459-460.
2. Centers for Disease Control and Prevention. H5N1 Bird Flu: Current Situation. https://www.cdc.gov/bird-flu/situation-summary/index.html. Accessed June 30, 2024.
3. Nguyen, T.Q., Hutter, C., et al., 2024. *Emergence and interstate spread of highly pathogenic avian influenza A (H5N1) in dairy cattle*. bioRxiv, pp.2024-05.
4. Stanislawek, W.L., Tana, T., et al., 2024. *Avian influenza viruses in New Zealand wild birds, with an emphasis on subtypes H5 and H7: Their distinctive epidemiology and genomic properties*. Plos one, *19*(6).
5. Bennison, A., Byrne, A.M., et al., 2023. *Detection and spread of high pathogenicity avian influenza virus H5N1 in the Antarctic Region*. bioRxiv, pp.2023-11.
6. Sahu, A., Kumar, N., et al., 2023. *Environmental DNA (eDNA): Powerful technique for biodiversity conservation*. Journal for Nature Conservation, 71.

PG37: Investigating the impact of fisheries on endangered hoiho diet, microbiome, and disease susceptibility

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Hoiho are classified as Nationally Endangered (NZCTS), but, with rapidly declining breeding numbers over the last 15 years on mainland Aotearoa their northern subpopulation could be functionally extinct within a few decades. Despite extensive conservation efforts to improve the status of the northern hoiho population, progress has been impeded by poor animal health. Diseases, including Diphtheritic Stomatitis, Respiratory Distress Syndrome, and Metabolic Bone Disease, have played a significant role in the declining numbers of adults and chicks in recent years. Research has also indicated a major change in hoiho diet has occurred. Whereas in the 1980s hoiho were feeding largely on small oily fish species such as sprat, immature red cod and āhuru; now blue cod makes up the majority of their diet[1]. The reasons for this change remain unexplained, but human fishing practices may have altered hoiho feeding habits and prey availability. The loss of important prey species could be playing a role in increased disease vulnerability in the northern population; affecting the microbiome, immunity, and overall health of the hoiho.

Metagenomic analysis of DNA extracted from hoiho faecal samples, collected from across all breeding locations of the northern and southern subpopulations of hoiho over an eight-year period (2017-2024), will enable the establishment of differences in diet and microbiome between subpopulations and locations, and any link to the cooccurrence of disease challenges at each location over this time frame. With the results, this research will help inform conservation management approaches to ensure the continued survival of hoiho across their entire range.

1. Young MJ, Dutoit L, Robertson F, van Heezik Y. 2020. *Species in the faeces: DNA metabarcoding as a method to determine the diet of the endangered yellow-eyed penguin.* Wildl Res 47(6): 509- 522.

PG38: Antibiotic resistance in Australian leafy greens pre-farm gate

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People all over the world consume fresh, uncooked horticultural produce, including leafy greens, for better health. However, consumption of contaminated raw produce increases the risk associated with foodborne disease outbreaks and exposure to antimicrobial-resistant bacteria (AMB) and antimicrobial resistance genes (ARGs) of clinical importance. There is a lack of both comprehensive data and standardised methods for accurate assessment of the microbiome and resistome in horticultural products. Metagenomic sequencing has been used to detect bacteria and ARGs in other sample types, such as human and animal clinical samples. However, there is limited application and benchmarks in horticulture, therefore this project focused on the development of a metagenomic sequencing pipeline to detect the presence of ARGs in the bacterial communities found in a lettuce microbiome.

Sample processing methods and shotgun metagenomic sequencing pipelines were developed and evaluated using glasshouse raised lettuce samples that were spiked with a mock community alongside a series of controls. The metagenomic data were analysed for the presence of the spiked bacteria and the carried ARGs. The detection limit of the bacteria and ARGs using the metagenomic approach were also examined. Two programs (SRST2[1] and Abricate[2]) were used to detect ARGs from the metagenomic reads and assembled contigs, respectively. The results indicate that a sequencing based metagenomic approach to investigating the microbiome lacked sensitivity to detect bacteria below 10^5 CFU/25g and any ARGs in bacteria below 10^6 CFU/25g. Due to the lack of sensitivity a more targeted method such as hybridisation capture will be explored as an alternative approach to detect bacteria and the carried ARGs in the leafy green microbiome.

1. Inouye, M., *et al.* (2014). *SRST2: Rapid genomic surveillance for public health and hospital microbiology labs*. Genome Medicine 6, 90.

2. Seemann, T. *Abricate*, Github https://github.com/tseemann/abricate

PG39: Sequential breakdown of the *Cf-9* leaf mould resistance locus in tomato by *Fulvia fulva*

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Leaf mould, caused by *Fulvia fulva*, is a devastating disease of tomato plants. During infection, *F. fulva* resides in the leaf apoplast, where it secretes virulence factors, termed effectors, to promote host colonization and disease. In resistant tomato cultivars, however, one or more of these effectors can be recognised by cognate cell surface-localised receptor-like proteins (RLPs) to trigger defence responses that halt *F. fulva* growth. In most commercial tomato cultivars, leaf mould resistance is governed by the *Cf-9* locus, which encodes the RLPs Cf-9C and Cf-9B. Of these, Cf-9C recognizes the previously identified Avr9 effector and provides resistance during all stages of plant growth, whereas Cf-9B recognises the yet-unidentified Avr9B effector and provides resistance in mature (flowering/fruiting) plants only. Over the last decade, *F. fulva* strains have emerged worldwide that can overcome the *Cf-9* locus, with *Cf-9C* circumvented through *Avr9* deletion. To understand how *Cf-9B* is circumvented, we set out to identify *Avr9B*[1]. Comparative genomics, transient expression assays and gene complementation experiments were used to identify *Avr9B*, with *Cf-9B* circumvention shown to be through gene deletion/mutation. Gene sequencing was used to assess *Avr9B* allelic variation across a worldwide strain collection. A strict correlation between *Avr9* deletion and resistance-breaking mutations in *Avr9B* was observed in strains recently collected from *Cf-9* cultivars, whereas *Avr9* deletion but no mutations in *Avr9B* were observed in older strains. This suggests that *F. fulva* has evolved to sequentially break down the *Cf-9* locus, with *Cf-9C* circumvented prior to *Cf-9B*.

1. de la Rosa, S., C.R. Schol, A. Ramos Peregrina, D.J. Winter, A.M. Hilgers, K. Maeda, Y. Iida, M. Tarallo, R. Jia, H.G. Beenen, M. Rocafort, P.J.G.M. de Wit, J.K. Bowen, R.E. Bradshaw, M.H.A.J. Joosten, Y. Bai and C.H. Mesarich (2024). *Sequential breakdown of the Cf-9 leaf mould resistance locus in tomato by Fulvia fulva*. New phytologist. https://doi.org/10.1111/nph.19925.

PG40: Loss of aminoarabinose lipid modification leads to cephalosporin antibiotic resistance in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic, multi-drug resistant pathogen, frequently causing respiratory tract infections in immunocompromised individuals. This bacterium is commonly found in the environment and hospital settings, causing infections in cystic fibrosis patient lungs, the urinary tract and burn wounds of immunocompetent patients. A particularly hypervirulent and multi-drug resistant strain of *P. aeruginosa* is the Liverpool Epidemic Strain LESB58. This pathogen utilises resistance mechanisms including an impermeable outer membrane and enzymes such as beta-lactamases which break down antibiotics. The polymyxin and cephalosporin classes of antibiotics are commonly used as last-resort treatments against complex multi-drug resistant *P. aeruginosa* infections. Polymyxins target and disrupt the bacterial cell membrane, while cephalosporins inhibit cell-wall synthesising enzymes. The *arnBCADTEF-ugd* gene cluster (*arn* operon; 9,380-bp) in *P. aeruginosa* encodes a lipid modification system that synthesizes an aminoarabinose sugar (aminoarabinosylation), neutralising the negative charge of the phosphate group of lipid A within the lipopolysaccharide (LPS) of the outer membrane. This modification repels positively charged polymyxin antibiotics and decreases their antimicrobial efficacy. We hypothesized that the deletion of the *arn* operon could result in resistance to other antibiotics targeting the bacterial cell wall. Antimicrobial susceptibility assays demonstrated that deletion of the *arn* operon resulted in increased resistance to the cephalosporin antibiotics ceftazidime (32-fold), cefepime (8-fold) and cefotaxime (8-fold). RNA-sequencing the *arn* mutant and wild-type strains following exposure to ceftazidime revealed significant upregulation of the beta-lactamase enzyme *ampC* within the *arn* mutant. Interestingly, overexpression of the gene encoding the *ampC* enzyme in the *P. aeruginosa* LESB58 wild-type strain only caused a moderate increase in resistance to the cephalosporins ceftazidime (4-fold) and cefepime (2-fold), indicating additional undiscovered mechanisms of resistance. Overall, our findings established the importance of the lipid modification system in cephalosporin resistance in *P. aeruginosa* LESB58, providing novel evidence of a previously undiscovered link between these resistance mechanisms.

PG41: Genetic diversity of *Mycobacterium tuberculosis* isolates circulating in Māori Communities

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Although Aotearoa has a low burden of tuberculosis, this burden is not evenly distributed through society. Māori continue to be affected by tuberculosis disproportionately compared to New Zealand Europeans. A symptom of this inequality could be driven by the unique *Mycobacterium tuberculosis* (Mtb) strain (informally known as Rangipo), which is strongly associated with Māori communities. During a Hawkes Bay outbreak of Mtb Rangipo in the 1990's, there were 94 infected cases with 14 of these cases having the active disease (15% of infection)[1]. This is three times more active cases than would be expected from a "normal" tuberculosis outbreak, suggesting Mtb Rangipo exhibits increased transmissibility.

Genome sequencing and phylogenetic analysis of the Rangipo strains identified two subvariants circulating in Māori communities. Bayesian divergence dating revealed an earlier divergence date for one subvariant that had not undergone clonal expansion. A closer investigation of the genomes identified a 12kb region containing genes implicated in immune evasion deleted in the subvariant that is associated with clonal expansion.

Taken together, the emergence of subvariants around the time of antibiotic adoption in the immediate post-war period, we hypothesise that this deletion may have allowed for enhanced transmission in an environment where improved, accessible treatments reduced the transmission of tuberculosis.

1. McElnay C, Thornley C, Armstrong R. *A community and workplace outbreak of tuberculosis in Hawke's Bay in 2002*. N Z Med J. 2004 Aug 20;117(1200):U1019.

PG42: Discovery and directed evolution of primordial chloramphenicol resistance genes from the soil metagenome

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Functional screening of the soil metagenome can provide insights into the prevalence of antibiotic resistance genes and potential new resistance mechanisms. This information helps predict resistance mechanisms that could emerge in clinical settings and informs stewardship measures. However, traditional screening methods have limitations: they can only transcribe and translate a minority of environmental genes, and they miss weaker “primordial” resistance genes that could evolve into high-level resistance with only a few mutations. To address these limitations, a novel pipeline was developed. This pipeline identifies primordial antibiotic resistance genes from the broader bacterial ‘resistome’ and then uses directed evolution to enhance resistance. By evolving these genes, researchers can assess the likelihood of resistance emerging in clinical settings. Identifying potential threats early allows for proactive testing of collateral resistance and sensitivity to other antibiotics, guiding responsible stewardship. Understanding enzyme-mediated resistance mechanisms can also aid in developing new generations of antibiotics that are not substrates for these enzymes. In this research, the pipeline was used to identify numerous primordial chloramphenicol resistance elements, with a gene annotated as a phosphotransferase being of particular interest. When tested for resistance to two newer generation amphenicols, thiamphenicol and florfenicol, the phosphotransferase conferred resistance to the former, but not the latter. NMR analysis has revealed the phosphotransferase can phosphorylate the primary hydroxyl group in thiamphenicol and chloramphenicol, a functional group that is absent in florfenicol. To assess the potential evolvability of the phosphotransferase, we conducted a directed evolution campaign. Variants with increased chloramphenicol resistance were tested for collateral resistance to thiamphenicol. Some variants conferred increased resistance to thiamphenicol, while others did not. These results indicate phosphorylation of chloramphenicol and thiamphenicol is a resistance mechanism that could potentially arise in a clinical setting, but that florfenicol could be used to counter this.

PG43: Comparison of nasal microbial secretion to middle ear effusion in preschool children with chronic otitis media with effusion

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Chronic otitis media with effusion (COME) in children can lead to prolonged hearing loss, which is associated with an increased risk of learning delays and behavioural problems. Spread of bacterial pathogens from the nasal passages to the middle ear is implicated in COME. We sought to compare the nasal and middle ear microbiomes to determine whether there is an association between nasal microbial composition and middle ear effusion composition in children with COME.

Nasal swabs and MEE were collected children aged 3 and 4 years with COME, and a questionnaire was administered to their caregivers. The V1-3 region of the 16S rRNA gene was amplified, and sequenced on the Illumina MiSeq.

Correlation of the bacteria present in the nasal passages and the middle ear may support a model of COME that includes transfer of pathogens between these microbiomes as a factor in the onset or maintenance of COME, with the nose and nasopharynx acting as bacterial reservoir. We will present our analysis of how the nasal microbiota may relate to the pathogenesis or maintenance of COME.