ID1: Germs & Genomics: Lessons learnt, unexpected connections and future pathways

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The rapid rise of genetic and genomic tools has enabled a precision and speed to pandemic science that has not previously been possible. Arguably, more so than in other countries during the COVID-19 pandemic, Aotearoa New Zealand has used many modes of genomic surveillance - from individuals and aeroplanes to locked-down cities and sewer sheds. This presentation will explore and describe decisions made and pathways travelled as the genomic toolkit was forced to respond, often rapidly, to the ever-changing pandemic landscape.

With a wider lens on the adoption of genetic and genomic tools, what can, or should, Aotearoa New Zealand do to advance its thinking, capability and education in this area? Importantly, how can we use the pandemic 'springboard' to promote and elevate the discussion of genetics/genomics with the wider community on the topic of infectious disease and beyond? Should we explore new pathways?

ID2: Vision Mātauranga and Research, Science and Innovation.

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The Vision Mātauranga policy was published in 2007 and has since become deeply imbedded in the New Zealand research funding system. Designed as a framework to unlock the distinctive innovation potential of Māori people, Māori knowledge, and Māori resources in Research, Science and Innovation, confusion remains for some researchers across the system as to how research projects can give life to Vision Mātauranga. There is also impetus to do so, given that funding criteria of major research funds now take into account how well applications address Vision Mātauranga. As the multi-year programme focussing on the future of New Zealand's research system - Te Ara Paerangi Future Pathways - moves forward, this presentation will reflect on the role of Vision Mātauranga in research and what lessons could be learned for the future of the research system.

ID3: Multi-antigen analysis of StrepA antibody responses reveals a distinct serological profile in children with acute rheumatic fever and sheds light on disease pathogenesis

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Streptococcus pyogenes (StrepA) causes superficial pharyngitis and skin infections as well as serious autoimmune sequelae such as acute rheumatic fever (ARF). ARF can progress to chronic rheumatic heart disease that is associated with significant morbidity and mortality in Māori and Pacific communities in New Zealand. Candidate vaccines that can prevent StrepA infections are being developed but have not yet reached licensure, and the limited understanding of ARF pathogenesis presents a major hurdle for the field. Immune priming by repeated GAS infections is thought to trigger ARF. There is also growing evidence for the role of skin infections in this process. We utilised our recently developed 8-plex immunoassay, comprising antigens used in clinical serology for the diagnosis of ARF (SLO, DNase B, SpnA), and five conserved prospective StrepA vaccine antigens (Spy0843, SCPA, SpyCEP, SpyAD, Group A carbohydrate), to characterise IgG antibody responses in sera from New Zealand children with a range of clinically diagnosed StrepA disease; ARF (n=79), StrepA pharyngitis (n=94), StrepA skin infection (n=51) and matched healthy controls (n=90). The magnitude and breadth of IgG antibodies in ARF was very high, with an average of 6.5 antigen-specific reactivities per individual observed, compared to 4.2 in skin infections and 3.3 in pharyngitis. The unique serological profile observed in ARF may be the result of repeated precursor pharyngitis and skin infections that progressively boost antibody responses. This highlights the importance of comprehensive ARF prevention strategies that target both StrepA pharyngitis and skin infections.

ID4: The bacterial pathogens *Listeria monocytogenes* and *Shigella flexneri* exploit host polarized exocytosis to enhance cell-to-cell spread

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Listeria monocytogenes and *Shigella flexneri* are evolutionarily unrelated enteropathogens that use an actin-based motility process to spread between cells in the human intestinal epithelium. Filamentous actin from the human cell forms a tail behind bacteria, propelling microbes through the cytoplasm. Motile bacteria remodel the host plasma membrane into protrusions that are internalized by neighboring cells. A critical unresolved question is whether generation of protrusions by *Listeria* or *Shigella* involves stimulation of host processes apart from actin polymerization. Here we demonstrate that efficient protrusion formation by each of these microbes requires bacterial stimulation of a host process called "polarized exocytosis" — the localized delivery of vesicles expand specific sites in the plasma membrane. Confocal microscopy imaging indicates that exocytosis is up-regulated in protrusions in a manner dependent on the host exocyst complex. Depletion of exocyst components by RNA interference (RNAi) inhibited the formation of *Listeria* or *Shigella* protrusions and subsequent cell-to-cell spread of these bacteria. Additional studies revealed that *Listeria* co-opts the human exocyst complex by using a secreted bacterial protein called InlC to recruit the exocyst component Exo70 to nascent bacterial protrusions. By contrast, *Shigella* uses a bacterial syringe-like structure called a type III secretion system (T3SS) to mobilize Exo70 the sites of protrusion generation. Depletion of Exo70 or other exocyst proteins decreased the lengths of *Listeria* or *Shigella* protrusions, indicating that the exocyst controls protrusion elongation. Collectively, these results indicate that two evolutionarily distinct bacterial pathogens target the human exocyst complex to enhance their intercellular dessimination in host tissues. We propose that exploitation of the exocyst by *Listeria* and *Shigella* may represent an example of convergent evolution.

ID5: A *Neisseria meningitidis* iron acquisition protein acts as an adhesin and inhibits host cell wound repair

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Neisseria meningitidis, or meningococcus, is the causative agent of meningococcal disease, a potentially fatal illness that can rapidly develop in previously healthy individuals. Meningococcus remains a top infectious killer globally; it can cause epidemics, lead to death within 24 hours, and leave one in five patients with lifelong disability after an infection. Meningococcal disease is estimated to be responsible for about 1.5% of total mortality in children under 5 years of age, and while infections with many strains of meningococci are vaccine-preventable, progress in preventing the disease lags behind that of other vaccine-preventable illnesses[1].

Although meningococci can cause a serious illness, in most instances the bacteria are carried harmlessly in the nasopharyngeal mucosa. The carriage state remains poorly understood, primarily due to lack of animal models for this human-adapted pathogen. Furthermore, epidemiological studies indicate that disruptions to the nasopharyngeal mucosa can increase the risk of invasive disease. Our previous work demonstrated that many meningococcal isolates were able to inhibit wound closure in a tissue culture model of wound healing[2]. We subsequently identified a meningococcal protein that has a role in haem acquisition and is required for inhibition of wound repair. Deletion studies and heterologous expression in a non-adherent bacterial host demonstrated that it is a key adhesin for epithelial cells. Our current studies aim to identify the host cell factor that it adheres to, and to identify the protein domains that are required for host cell adherence.

1. GBD 2016 Meningitis Collaborators (2018) *Global, regional, and national burden of meningitis, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016.* Lancet Neurol 17:1061-82.

2. Ren, X. and MacKichan, J.K (2014) *Disease-associated Neisseria meningitidis isolates inhibit wound repair in respiratory epithelial cells in a Type-IV pilus-independent manner.* Infect Immun 82(12): 5023-34.

ID6: Novel insights into the structure and function of dengue virus NS1 protein

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Flaviviruses infect almost 400 million people per year and cause a number of severe diseases. The dengue flavivirus (DENV) is responsible for dengue fever. While generally mild, a small proportion of cases develop into dengue haemorrhagic fever or dengue shock syndrome, which can be life-threatening. Flaviviruses have a positive-strand RNA genome that encodes a single, large polyprotein, which is processed into three structural and seven non-structural proteins. Six of the non-structural proteins form the viral genome replication complex. While the remaining non-structural protein, NS1, is essential to flavivirus genome replication, it has no known catalytic function. Early in infection, dimeric NS1 localises on the endoplasmic reticulum membrane at the site of viral RNA replication and may be involved in formation of the replication complex. Late in infection, NS1 is secreted as a hexameric lipoprotein and interacts with components of the complement-mediated immune system, with the level of NS1 in serum correlating with the onset of dengue hemorrhagic fever.

In 2014, two crystal structures of full-length, glycosylated[1], and truncated[2] West Nile Virus (WNV) and Dengue Type 2 Virus (DENV2) NS1 dimers were obtained, which have aided interpretation of two low-resolution cryo-electron microscopy reconstructions of the hexameric structure of secreted NS1[3,4]. We have leveraged this structural information along with biochemical and NMR analyses of the NS1 lipid cargo[4] to build and simulate models of the DENV2 NS1 hexamer both with and without a lipid cargo. Coarse-grained molecular dynamics simulations showed that the glycosylation of NS1 is essential for hexamer stability, with the glycans compensating for the paucity of protein-protein contacts between dimers. NS1 hexamers pre-loaded with a lipid cargo can pick up lipids from their environment and can deposit lipids into membranes but are not able to pick up lipids from membranes, whereas NS1 hexamers without a lipid cargo are not as effective at picking up environmental lipids. The lipid cargo forms a dumbbell shape, with polar lipids towards the outside of the bulbs and non-polar lipids in the centre of the bulbs or the bar of the dumbbell. These results add to our somewhat scarce understanding of the otherwise enigmatic function of a protein whose presence is an important biomarker for dengue fever, but whose role in infection has been controversial and is not well understood.

ID7: Application of genomic epidemiology and evolutionary modelling for the control of human, animal and environmental pathogens in Aotearoa New Zealand

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Genomic epidemiology has played an important role in the control of the COVID-19 pandemic in New Zealand. Sequencing of SARS-CoV-2, combined with evolutionary modelling enabled the impact of the elimination strategy on the first wave of the pandemic to be evaluated[1] and, by delineating post-elimination outbreaks in near-real time and determining transmission chains, supported decision making for the management of subsequent cross-border incursions and outbreaks[2-4]. Although not in real time, genome sequencing and the application of ancestral state reconstruction methods have also supported the response towards the elimination of *Mycoplasma bovis* from the cattle population in Aotearoa. Related approaches have been used for the control of foodborne and environment pathogens; including antimicrobial resistant *Campylobacter jejuni*, S*almonella enterica* and *Yersinia pseudotuberculosis*.

In this talk I will summarise how genomic epidemiology has been applied for the control of multiple human, animal and environmental pathogens in Aotearoa. I will demonstrate how ancestral state reconstruction models, multivariate methods and distance-based linear models, combined with visualisation tools such as Nextstrain[5] and Microreact[6], have been used to understand transmission patterns and inform control strategies at different spatial and temporal scales. The opportunities and pitfalls of using genome sequence data to determine the source of human infection and environmental contamination will be described, with reference to the control of foodborne and environmental pathogens.

1 Geoghegan, J. L. *et al.* (2020) *Genomic epidemiology reveals transmission patterns and dynamics of SARS-CoV-2 in Aotearoa New Zealand*. Nat Commun 11, 6351, doi:10.1038/s41467-020-20235-8.
2 Jelley, L. *et al.* (2022) *Genomic epidemiology of Delta SARS-CoV-2 during transition from elimination to suppression in Aotearoa New Zealand*. Nature Communications 13, 4035, doi:10.1038/s41467-022-31784-5.
3 Geoghegan, J. *et al.* (2021) *Use of Genomics to Track Coronavirus Disease Outbreaks, New Zealand*. Emerging Infectious Diseases 27, doi:10.3201/eid2705.204579.
4 Douglas, J. *et al.* (2021) *Real-Time Genomics for Tracking Severe Acute Respiratory Syndrome Coronavirus 2 Border Incursions after Virus Elimination*, New Zealand. Emerging Infectious Diseases 27, 2361-2368, doi:10.3201/eid2709.211097.
5 Hadfield, J. *et al.* (2018) *Nextstrain: real-time tracking of pathogen evolution*. Bioinformatics 34, 4121-4123, doi:10.1093/bioinformatics/bty407.
6 Argimón, S. *et al.* (2016) *Microreact: visualizing and sharing data for genomic epidemiology and phylogeography*. Microbial Genomics 2, e000093, doi:10.1099/mgen.0.000093.

ID8: Tracking SARS-CoV-2 spread and evolution through genomic sequencing

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Nextstrain is a collaborative open-source project to enable real-time phylogenetic analysis of pathogen genomes. Since January 2020 we have been running near-daily analyses of publicly available SARS-CoV-2 genomes, which now number in the millions. This talk will revisit our initial datasets, where the focus was on geographical spread, and move through to our current work which is predominantly concerned with detecting and tracking variants. I will compare the antigenic evolution of recent months with that of influenza and seasonal coronaviruses, and what this may mean for vaccine updates. Finally, I will touch on where this leaves us for future pathogen outbreaks both in New Zealand and elsewhere now that “genome sequencing” is in common parlance.

ID9: Genome evolution drives transcriptomic and phenotypic adaptation in *Pseudomonas aeruginosa* during 20 years of infection

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The opportunistic pathogen *Pseudomonas aeruginosa* chronically infects the lungs of patients with cystic fibrosis (CF). During infection the bacteria evolve and adapt to the lung environment. Here we examine genomic changes, and consequent transcriptional and phenotypic changes, in isolates of a *P. aeruginosa* lineage from a CF patient over more than 20 years of chronic infection. Complete genome sequencing of the isolates showed that a genetic bottleneck occurred during infection and was followed by diversification of the bacteria. A 127 kb deletion and hundreds of smaller mutations occurred during evolution of the bacteria in the lung, with an average rate of 17 mutations per year. Many of the mutated genes are associated with infection or antibiotic resistance. RNAseq showed that extensive reprogramming of the transcriptional network occurred during infection, affecting multiple genes. Changes included greatly reduced expression of motility-associated genes and increased expression of genes for nutrient acquisition and biofilm formation, as well as altered expression of a large number of genes of unknown function. Phenotypic studies showed that most later isolates had increased cell adherence and antibiotic resistance, reduced motility, and reduced production of pyoverdine an iron-scavenging siderophore, consistent with genomic and transcriptomic data. Our findings reveal, and help to explain, the extent and effects of genomic changes that *P. aeruginosa* undergoes as it adapts to the environment of the CF lung during a chronic infection

ID10: Genomic landscape of meningococcal disease in post-epidemic New Zealand.

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In 2017 the World Health Organisation issued a call to eliminate meningitis globally by 2030. A draft global road map[1] published in October 2020 proposed enabling a fast, accurate and efficient global surveillance system as a key component of the road map. New Zealand has been conducting genomic surveillance for *Neisseria meningitidis* since 2017 and contributes to the global surveillance by participating in the Invasive Respiratory Infections Surveillance programme.

Between 1991 and 2007, New Zealand experienced a prolonged epidemic of meningococcal disease, with the majority of cases caused by a single group B, PorA type P1.7-2.4 strain (NZMenB). To control the epidemic, a strain-specific vaccine (MeNZB^{TM}) was rolled out in stages from 2004 to all aged less than 20 years. Following vaccine introduction there was a decrease in meningococcal cases and the vaccination programme ended in 2008.

We will describe the epidemiology of invasive meningococcal disease post epidemic using notification data extracted from the New Zealand national notifiable disease surveillance database (EpiSurv). We will present a comprehensive analysis of invasive meningococci isolates in New Zealand between 2013 and 2020 using whole-genome sequencing. We describe the population structure of meningococci following the NZMenB epidemic and examine the clonal distribution of circulating isolates over time. We also present evidence a new variant of the group W colonal complex 11 strain, which is different from the strain responsible for disease increases in Europe, is responsible for an increase of invasive disease in New Zealand since 2015[2]. In addition, we will discuss how the COVID-19 pandemic and public health measures impacted meningococcal disease in New Zealand[3].

1. *Defeating meningitis by 2030: a global road map* https://cdn.who.int/media/docs/default-source/immunization/meningitis/defeatingmeningitisroadmap.pdf?sfvrsn=74ae28ce_13&download=true
2. Yang Z, Ren X, Davies H, Wood T, Lopez L, Sherwood J, et al. *Genomic Surveillance of a Globally Circulating Distinct Group W Clonal Complex 11 Meningococcal Variant, New Zealand, 2013–2018.* Emerg Infect Dis. 2021;27(4):1087-1097. https://doi.org/10.3201/eid2704.191716
3. Brueggemann AB et. al, *Changes in the incidence of invasive disease due to Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data.* Lancet Digit Health. 2021 Jun;3(6):e360-e370. doi: 10.1016/S2589-7500(21)00077-7.

ID11: The 'Midnight' Method: Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using Oxford Nanopore Rapid Barcoding

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Rapid and cost-efficient whole-genome sequencing of SARS-CoV-2, the virus that causes COVID-19, is critical for understanding viral transmission dynamics. We show[1] that using a multiplexed set of primers in conjunction with the Oxford Nanopore Rapid Barcode library kit allows for faster, simpler, and less expensive SARS-CoV-2 genome sequencing. This primer set we developed results in amplicons that exhibit lower levels of variation in coverage compared to other commonly used primer sets and has held up well despite the emergence of new variants. We show that high-quality genomes can be generated with as few as 10,000 reads (~5 Mbp of sequence data). This method enables the user to go from patient sample to fully assembled genome in approximately 8-9 hours. This method cuts the time from RNA to genome sequence by more than half compared to the more standard ligation-based Oxford Nanopore library preparation method at considerably lower costs. Our method, dubbed the "Midnight" method is currently in use in over 35 countries, at over 180 organisations. It has been scaled up and packaged for distribution by IDT and Oxford Nanopore Technologies. The protocol has been accessed over 37,000 times and is a leading method worldwide for sequencing the virus.

1 .Nikki E Freed, Markéta Vlková, Muhammad B Faisal, Olin K Silander, *Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using 1200 bp tiled amplicons and Oxford Nanopore Rapid Barcoding,* Biology Methods and Protocols*,* Volume 5, Issue 1, 2020, bpaa014, https://doi.org/10.1093/biomethods/bpaa014

ID12: Genetic diversity and transmission patterns of *Mycobacterium tuberculosis* in low and high tuberculosis burden countries

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Tuberculosis (TB) is a curable disease caused mainly by the bacterium *Mycobacterium tuberculosis* (Mtb), and yet paradoxically it claims 5000 lives daily. Of growing concern is multidrug-resistant TB (MDR-TB) and extensively drug-resistant (XDR-TB) being the leading cause of deaths related to antimicrobial resistance (AMR). Despite Aotearoa New Zealand being a low TB burden country, Māori and Pasifika are disproportionally affected by TB compared to New Zealand Europeans. Remarkably, there are Mtb strains endemic to Māori and Pasifika communities. On the other hand, Myanmar, one of 10 countries listed by the World Health Organization WHO for the high burden of TB, TB and HIV, and MDR-TB, has diverse Mtb strains. In this talk, I will discuss how we are using whole-genome sequencing (WGS) to unravel genetic diversity and transmission patterns of Mtb strains in Aotearoa (low burden setting) and Myanmar (high burden setting), and translating this WGS-guided evidence to TB healthcare policymaking and routine management.

ID13: Point-of-Care technology for infectious disease control

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The COVID-19 pandemic demonstrates the fragility of global human health systems. Important tools in the fight to contain disease spread are accurate, robust and appropriate diagnostics to rapidly detect and trace outbreaks. At the onset of the SARS-CoV-2 outbreak RT-qPCR based testing presented the best method for diagnosing infection. In traditional laboratory settings RT-qPCR requires large and expensive specialised equipment and skilled practitioners. However, a centralised laboratory process brings with it requirements for concentrating people at central collection points, transport of infectious material and delayed time-to-result for potentially infectious individuals. Current advances in viral nucleic acid extraction, liquid handling and qPCR devices present viable options for point-of-care approaches to screen people in situ and deliver immediate results. Implementing in-field screening for infectious disease potentially creates safer, more controlled environments that could permit economic and cultural activities to continue more normally throughout a pandemic. This address will present our work to develop sample-to-answer point-of-care diagnostic systems that meet operational requirements suitable for imbedded community–based routine infectious disease testing.

ID14: From Poop to PCR: Wastewater Surveillance of SARS-CoV-2 in Aotearoa

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Wastewater testing for SARS-CoV-2 RNA provides a reliable and sensitive platform for detecting infections at the community scale and has played a key role in Aotearoa New Zealand's response to the pandemic. Wastewater surveillance during the period in which an elimination strategy was pursued helped to identify and/or delimit occasional outbreaks, allowing escalation of targeted public health measures when and where needed. Wastewater surveillance activities scaled up significantly in August 2021 as the Delta variant arrived in the country.

For the last 12 months, wastewater testing has covered over 70% of New Zealanders connected to reticulated wastewater (up to 3.8million people). Viral titres in wastewater correlated strongly with number of clinically defined COVID-19 cases contributing to the sample[1], particularly before the widespread reliance on self-reported RAT results to determine case numbers. Since early 2022, RATs have become an increasingly common COVID-19 community tracking tool. As a result, case under-reporting has become marked and wastewater surveillance has gained prominence in monitoring disease trends.

Wastewater surveillance is also used to track SARS-CoV-2 variants circulating at sentinel sites each week, revealing successive and rapid take-overs by newer variants. We describe efforts to identify variants using targeted assays and whole-genome sequencing.

Wastewater-based epidemiology to monitor SARS-CoV-2 has provided a new surveillance platform in Aotearoa, and has been successfully used to detect outbreaks, monitor trends and track variants. This platform will be expanded this coming year to cover other infectious human pathogens of public health concern.

1. Hewitt, J., Trowsdale, S., Armstrong, B. A., Chapman, J. R., Carter, K. M., Croucher, D. M., Trent, C. R., Sim, R.E. and Gilpin, B. J. (2022) *Sensitivity of wastewater-based epidemiology for detection of SARS-CoV-2 RNA in a low prevalence setting.* Water Research 211: 118032.

ID15: Development of mRNA vaccines that induce liver-resident memory CD8$^+$ T cells that protect against hepatotropic infection

Mitch Ganley[1], Lauren Holz[2], Sarah Draper[1], Regan Anderson[1], Benji Compton[1], Andrew Marshall[1], Olivia Burn[3], Jordan Minnell[3], Ian Hermans[3,4], William Heath[2], Gavin Painter[1,4]

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We are particularly interested in the generation of a sub-population of non-circulating memory T cells known as liver resident-memory CD8$^+$ T cells (T_{RM} cells), and their role in two important diseases of the liver, malaria and hepatitis B (HB). Antigen-specific liver T_{RM} cells have been touted as 'from-line defenders' against *Plasmodium* sporozoite infection, which causes malaria, and have been implicated in the clearance of HB infection from human liver cells. Although an anti-malarial vaccine (Mosquirix) was recently approved, this treatment only achieves ~30% protection after 1 year and drops to <10% protection after 4 years.

Additionally, there are no therapeutics for treating chronic HB (cHB) infection. In Aotearoa it is estimated that 100,000 people are chronically infected with HB, with Māori and Pasifika being six times more likely to be impacted by HB infection. Whilst current anti-viral therapy reduces viremia, its long-term benefit wanes, with life threatening liver cirrosis and cancer rates significantly increasing with long-term use These therapies can also be expensive (~$15,000/yr) and generally do not reach those most at risk.

We will discuss the design, construction and preclinical testing of mRNA vaccines for the induction of T_{RM} cells in the liver and our progress towards the development of efficacious vaccines for malaria and cHB.

ID16: Teaching the unteachable: training innate immune cells in the lung using the BCG vaccine

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Mycobacterium bovis bacille Calmette-Guérin (BCG) is the vaccine used to prevent tuberculosis (TB) and protective in only ~50% of cases when delivered intradermally. Beyond its effects on TB, BCG is known to protect mice against a wide range of unrelated infectious organisms, and in human infants can prevent all-cause mortality and in mice and humans it can even lead to cancer regression. These pluripotent effects are thought to be mediated by training of innate immune cells that allow them to respond more effectively to TB, other infectious diseases and cancers. Although cells of the innate immune were thought to be 'fixed' in their responsiveness, BCG-exposed innate cells have been shown to increase their ability to produce key effector functions to inhibit pathogen growth and survival, including pro-inflammatory cytokines and reactive oxygen species. Since TB is a pulmonary infection, we sought to measure the effects of BCG on the innate immune cells in the lung by administering the vaccine intranasally to mice. We found that the BCG vaccine increases the frequencies and numbers of lung macrophage subsets, and notably increase the proportion of alveolar macrophages expressing CD11b prior to and following a challenge infection. *In vitro* mycobacterial growth inhibition assays revealed that sorted CD11b-expressing alveolar macrophages have a greater capacity to inhibit mycobacterial growth than conventional CD11b-low alveolar macrophages. Furthermore, transcriptomes from CD11b-high alveolar macrophages sorted from lungs of BCG-vaccinated mice were found to be enriched for transcripts encoding mycobacterial receptors, activation receptors, molecules and receptors involved with antigen presentation, chemokines, and interferon responsiveness. These data suggest that BCG training of lung macrophages may contribute to the enhanced protection against infectious challenge that is observed in BCG-immunised mice.

ID17: From Covid-19 to monkeypox – issues for vaccine safety and confidence

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Necessity is the mother of invention. In response to the covid-19 pandemic the obstacles to taking a vaccine from early human trials to large scale efficacy studies, upscaling of production and mass deployment without skipping steps, were removed. Science was ready but public health was not. The mass vaccination of the global population is a massive undertaking and includes not only getting vaccines into arms fast and efficiently but also responding to the associated infodemic, which includes disinformation and misinformation. The issue is not new and for over two centuries the most commonly cited concern about vaccines, albeit in many guises, has revolved around the perceived safety. Therefore the generation of data and its effective communication is a vital component for a successful vaccine programme.

Today we face a new emerging infectious disease challenge. Monkeypox has established itself in communities far from its endemic origin in West Africa and is spreading fast. We have the tools to shut the spread of this virus down, including public health antivirals and vaccines developed over the last forty-years in the event of a potential smallpox threat.

Covid-19 and monkeypox are very different infectious diseases in most respects but there are two challenges they share when it comes to vaccination. One is real and perceived vaccine safety issues and the other is misinformation. This talk will be about how vaccine safety is established, what we will be doing to assess monkeypox vaccines, and the challenges for communicating this.

ID18: Exploring the cross-reactive immune response to *Neisseria gonorrhoeae* using MeNZB antisera

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Neisseria gonorrhoeae, the causal agent of gonorrhoea, is a bacterium of global concern due to its rapid development of antimicrobial resistance. There is no vaccine and progress has been confounded by the lack of known correlates of protective immunity. The finding that New Zealand's MeNZB vaccine, which contains Outer Membrane Vesicles (OMVs) from *Neisseria meningitis* serogroup B (MenB), has a vaccine efficacy of 31% to *N. gonorrhoeae*[1] has stimulated renewed hope for a vaccine. MeNZB OMVs are an important component of new broadly protective licenced vaccine (Bexsero) for MenB.

Paired baseline and immune sera (at least n=20 per assay) from pre-teen participants in Phase II clinical trials of MeNZB were used to examine potential cross-reactivity to *N. gonorrhoeae* and results compared by two-tailed Wilcoxon matched-pairs signed rank test. Endpoint titre ELISAs using OMVs from international reference strains of *N. gonorrhoeae* (FA1090, P9-17 and MS11) indicate that participants developed moderately increased IgG titres after vaccination (P9-17 $p<0.05$; FA1090, MS11 $p<0.001$). Serum Bactericidal Antibody (SBA) is a key correlate of protective immunity against MenB. MeNZB vaccination stimulated a small increase in SBA-mediated killing of *N. gonorrhoeae* MS11 (median=6.7%, $p<0.0001$) and FA1090 (median=17%, $p<0.0001$) but not P9-17 (median=0), but these differences are unlikely to be biologically meaningful. Reduced adherence of *N. gonorrhoeae* to the genital epithelial cell line ME-180 in the presence of MeNZB anti-sera was strain dependent: MS11, median = 20%, $p<0.0001$ compared with P9-17, median = 3%.

Vaccination with MeNZB OMVs stimulates a modest cross-reactive response to *N. gonorrhoeae*, including the potential to diminish adherence to a target cell line. Additional isolates will be applied to these assays and to antigen-identification studies to gain a more complete picture of the contribution of antibody-mediated responses targeting *N. gonorrhoeae*. This information contributes to international efforts towards developing a vaccine for gonorrhoea.

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ID19: Investigating the roles of unique Pacific gene variants on innate immunity and susceptibility to viral pathogens

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Numerous infectious diseases including influenza and measles were introduced into infection-naïve Pacific populations between the 16th and 19th centuries, decimating indigenous communities. First contact disease mortality estimates include 80% of the Marquesas population between 1791-1864 from typhoid, tuberculosis, influenza and smallpox (1), and 94% of the Hawaiian Islands population between 1778-1900 from waves of novel infectious diseases (2).

Evidence demonstrating the impact of host genetic variants on the immune response is steadily growing with up to three quarters of immune traits attributed to genetic factors (3). Survival of historical infectious diseases that swept across the Pacific region may have relied on a pathogen-resistant phenotype, effectively selecting for more resistant genotypes. Pacific peoples have a significant number of unique genetic variants for which the impacts on immunity and pathogen susceptibility are unknown.

This study aims to investigate unique genetic variants identified from sequencing data at minor allele frequencies over 10% in Western Pacific populations but absent in Europeans, to find potential links to altered immune responses or susceptibility to viral infection. From 1,835 coding variants, genes were manually filtered for established links to the innate immune response and viral susceptibility to select a shortlist of variants for further examination.

CRISPR-Cas9 gene editing was used to introduce the shortlisted Pacific gene variants into permissible cell lines to be studied for impacts on susceptibility to different viruses in cell culture. Viruses to be examined in Pacific variant-bearing cell lines include influenza A virus H1N1, *Herpes simplex* virus and human coronavirus OC43, compared to the reference cell lines. Additionally, PBMCs from relevant Pacific variant carriers will be tested for altered cytokine production and compared to non-Pacific-derived PBMCs. This study aims to identify potential links between unique Pacific gene variants and impacts on the early innate immune response to viral pathogens.

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ID20: Yeast-based production of SARS-CoV-2 spike RBD for vaccine development

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The ongoing COVID-19 pandemic still represents a serious threat to human health with over 1000 covid-related deaths per day worldwide. Long lasting effects of serious Covid-19 infection and the critical ability of the virus to adapt to the human host aggravate the global burden and have caused a pressing need for research into fast and effective vaccine development and production. The SARS-CoV-2 virus particle can infect human tissues containing the angiotensin-converting enzyme 2 (ACE2) surface protein. The direct interaction between the receptor-binding domain (RBD) of the viral spike protein (S) and ACE2 represents the first critical step in the viral host cell invasion, which can be blocked by anti-RBD antibodies. The elucidation of the SARS-CoV-2 spike protein[1] allowed for the targeted design of functional subunit vaccines[2] that aim specifically at the generation of these neutralising antibodies to create a lasting protective immune response.

Here we present the successful production of functional SARS-CoV-2 spike RBD in the methanotrophic yeast *Pichia pastoris* (*Komagataella phaffii*) as a simple, unmodified construct. Homologous expression of viral spike RBD in yeast[3] is advantageous due to its easy growing conditions and high scalability[4] while retaining post-translational processing capabilities. The highly pure RBD was shown to be an effective stimulant of neutralising serum antibody levels in a mouse model and represents a simple scaffold for further protein engineering and modification to optimise the overall immunological response. This work, performed in conjunction with the Vaccine Alliance Aoteroa New Zealand - Ohu Kaupare Huaketo, aims to strengthen the local capability to develop and produce vaccines at pace.

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ID21: The global antimicrobial resistance crisis: where does New Zealand stand?

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Antimicrobial resistance is a global concern. By international standards, infection rates due to antibiotic-resistant organisms in New Zealand are comparatively low. However some strains of antibiotic-resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) and isolates expressing an extended spectrum beta-lactamase (ESBL) are well established in New Zealand.

The Antibiotic Reference Laboratory at ESR is responsible for the national surveillance of antimicrobial resistance among human pathogens and provides a reference service for the confirmation and characterisation of antimicrobial resistance. Isolates are characterised using a combination of traditional phenotypic methods, molecular-based assays and whole genome sequencing. Important and emerging resistant bacteria are targeted for enhanced surveillance.

The most serious antibiotic resistant threat to New Zealand are carbapenemase-producing Enterobacterales (CPE). Carbapenemase production enables bacteria to grow in the presence of broad-spectrum carbapenem antibiotics, which are considered to be the last resort for treating serious Gram-negative bacterial infections. Historically New Zealand patients with CPE are likely to have acquired their CPE overseas although there are an increasing number of cases where acquisition in New Zealand is suspected.

ID22: From antimicrobial stewardship to renewal

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Those who coined the term 'antimicrobial stewardship' "wanted to highlight that we should consider antimicrobials a precious non-renewable resource"[1]. Once resistance to a drug emerges in a species that had been uniformly responsive, it never seems to fully disappear again. What would we have to do to grow back microbial ecosystems in which resistance had to evolve again, and antimicrobials could be gifted to future generations? This presentation will be about the potential of an island nation to be a refuge for susceptibility. I'll discuss our surveys of resistance using both urban and rural waterways and mahinga kai as physical and biological sentinels. Then I will summarise new work on the broader chemical exposome for microbes, particularly pesticides and emulsifiers (surfactants) and how these can act to accelerate resistance to antibiotics in short term evolution experiments. Bees, trees and humans incubate resistance in the chemical age, making the microbiological world resistance ready. In this broader context, narrow definitions of stewardship may lead to underestimation of the challenge of resisting resistance.

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ID23: Biofilm infections are insensitive to antibiotics; how can they be eradicated?

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Biofilms are often found at surfaces and comprise a heterogenous population of microbes within a polymeric matrix. Biofilm infections, where biofilms grow on tissue or implanted devices, are reported to be responsible for about 65% of all infections and are especially associated with chronic infections. Biofilms exhibit high levels of antibiotic tolerance and without specific resistance genes can be 1000× less sensitive to an antibiotic than cells in broth cultures grown from the biofilm bacteria. This means there can be a substantial difference between the antibiotic sensitivity of the biofilm causing the infection and the cells derived from that biofilm used for sensitivity testing in the laboratory. Biofilms therefore are often a factor in antibiotic treatment failure and new therapies are needed to target biofilms. Specifically, there is a need for drugs that can penetrate or disrupt the polymeric matrix and provide efficacy against the phenotypically variable cells within the biofilm, including the recalcitrant persister cell population.

Lactoferrin is an iron-binding protein from the innate immune system, with bovine milk providing an abundant source. The multifunctional nature of Lactoferrin has suggested many potential therapeutic applications, including as an antibiofilm antimicrobial. We have combined bovine Lactoferrin with conventional antibiotics to test antibiofilm effectiveness. We demonstrate that Lactoferrin can act as a potentiating antibiotic adjuvant to eradicate mature bioreactor-grown *Staphylococcus aureus* biofilms with beta-lactam antibiotics that are active against broth grown cells, but ineffective against biofilm, when used alone. Our findings offer evidence to support the wider evaluation of Lactoferrin as an antibiofilm strategy, especially given the long safety history that makes it a viable therapeutic candidate.

ID24: Novel inhibitors for combating obligate anaerobic pathogens

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Anaerobic bacteria abundant in the mammalian gastrointestinal tract are essential for human and animal health. However, imbalances in this microbial community favouring opportunistic anaerobic pathogens can lead to dysbiosis and disease. New therapeutic strategies are, therefore, required to inhibit the growth of anaerobic pathogens while leaving the commensal bacteria intact. As such, there has been much interest in exploiting metal ion (i.e., zinc) binding compounds as antimicrobials due to the vital role metal ions have in bacterial physiology. Recently, the zinc ionophore PBT2 has been demonstrated to have potent antibacterial activity *in vitro* and in *in vivo*. Additionally, natural products still play an essential role in the antibiotic discovery pipeline. To identify novel antimicrobials against anaerobic pathogens, we focused our investigation on *Fusobacterium nucleatum* and *Clostridiodes difficile.*
F. nucleatum is associated with cancer disease progression and inflammatory bowel disease, and the development of specific *Fusobacterium* inhibitors has been proposed for treating colorectal cancer. *Clostridiodes difficile* infection is the leading cause of nosocomial antibiotic-associated diarrhoea, and novel antibiotics are required to target *C. difficile* but not the resident host microbiota.

Using antimicrobial susceptibility testing, ICP-MS, RNAseq and qRT-PCR, we characterised the physiological and transcriptional response of *F. nucleatum* ATCC 25586[1] to PBT2-Zn. We demonstrated that PBT2-Zn exhibited potent antimicrobial activity by destabilising intracellular metal ion homeostasis. Further, to identify a new chemical target space for antimicrobial development and to explore the physiology of *C. difficile,* we performed high-throughput screening of a natural product library against vegetative cells of the non-toxigenic isolate *C. difficile* ATCC 700057. Selected plant-derived compounds anacardic acid, acetyl-11-keto-ß-boswellic acid (AKBA), and isobavachalcone were investigated for their antimicrobial activity against *C. difficile in vitro*[2].

This work provides new insights into how these anaerobic pathogens respond to metal ion stress and establishes bacterial metallostasis as a promising anaerobic drug target.

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ID25: Peptides and peptidomimetics to combat adaptive multidrug resistant high-density infections

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There has been enormous publicity about the inexorable rise of antibiotic resistance and the dearth of new therapies. However less attention has been placed on adaptively multidrug resistant high-density bacterial infections. Intractable infections such as typified by abscess infections are responsible for millions of annual emergency room visits around the world and although antibiotics are highly used, no effective therapies currently exist. Here we show how peptides and peptoids (peptidomimetics) enhance the activity of antibiotics and can be used to treat bacterial biofilms and high-density infections caused by the multidrug resistant pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We employed a bacterial, subcutaneous abscess mouse model to demonstrate that peptides can be used to pharmacologically treat infections and to reduce the severity of cutaneous abscesses. Adjuvant therapy with clinical antibiotics further reduced abscess lesions and bacterial loads. Peptoids are isomerically related to peptides with side chains appended to the amide backbone nitrogen rather than the α-carbon atom and are more appealing as therapeutics due to their decreased susceptibility to proteolysis and relatively lower synthesis costs. We investigated several structurally-related peptoids to treat *P. aeruginosa* and *S. aureus* biofilms, individually (monomicrobial) and combined (polymicrobial), under host-mimicking conditions. Using our new *Pseudomonas-Staphylococcus* co-infection skin abscess mouse model, we showed that under these challenging conditions peptoids were able to reduce the severity of the co-infection. These peptides and peptoids have therefore the potential to broaden our limited antibiotic arsenal for extremely difficult to treat infections caused by multidrug (antibiotic) resistant pathogenic bacteria.

ID26: BD oxidase is a game-changing drug discovery target for tuberculosis

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Tuberculosis (TB) remains a leading worldwide cause of death from infectious diseases with over 1 million annual deaths. And the spread of drug-resistant strains threatens to return us to the pre-antibiotic era for this disease. However, *bd* oxidase, the terminal oxidase from *Mycobacterium tuberculosis* has emerged as an exciting new drug target with the potential to be used in new treatment regimens that could rapidly cure tuberculosis infection in weeks rather than months, as is now required.

Structure determination of a target protein, like *bd* oxidase, is recognised as a key step in the development of strong and specific inhibitors but the structure of *bd* oxidase from mycobacteria has been a tough nut to crack. However, by employing advanced expression and cryo-electron microscopy methodology we have been able to determine the structure of this key membrane protein. This 2.5Å structure was found to differ in several key ways from the other bd structures. Specifically an extra substrate binding site was identified near haem b_{595}, new oxygen-binding and water channels were identified, and an unexpected disulfide bond was found which restricts the mobility of the substrate binding Q-loop. All of these findings have the potential to alter substrate binding and will need to be incorporated into structure-aided drug discovery efforts, which if successful could dramatically impact the clinical treatment of tuberculosis.

Summary of Abstracts for the Poster Session

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ID27	A Novel Recombinant *Lactococcus lactis* Mucosal Vaccine Platform Based on Group A Streptococcus Pili	Tsai, C.J-Y.[1,2], Blanchett, S.S.[3], Loh, J.[1,2], Proft, T.K.[1,2]	[1]*Department of Molecular Medicine & Pathology, University of Auckland,* [2]*Maurice Wilkins Centre, NZ,* [3]*Division of Infection & Immunity, Faculty of Medical Sciences, University College London, UK.*	37
ID28	Bacterial competence or biofilm formation? Potential roles of the gonococcal periplasmic ligase, LigE.	Pan, J.[1], Hicks, J.[1], Williamson, A.[1]	[1]*School of Science, University of Waikato*	38
ID29	Developing an mRNA vaccine for malaria	Mitch Ganley[1,2]	[1]*Ferrier Research Institute, Victoria University of Wellington* [2]*Malaghan Institute of Medical Research, Wellington*	39
ID30	Movement for Change: Structural Rearrangement and Interaction of the Transcriptional Regulator CysB with DNA	Klaus, H.[1] Oldham, K.[1], Arcus, V.[1] & Hicks, J.[2]	[1]*School of Science, University of Waikato,* [2] *Te Huataki Waiora, School of Health, University of Waikato*	40
ID31	Cloacal virome of an ancient host lineage – the tuatara (*Sphenodon punctatus*) – reveals abundant and diverse diet-related viruses	Stephanie J. Waller[1], Sarah Lamar[2,3], Benjamin J. Perry[1], Rebecca M. Grimwood[1], Edward C. Holmes[4], Jemma L. Geoghegan[1,5]	[1]*Department of Microbiology and Immunology, University of Otago,* [2]*School of Biological Sciences, Victoria University of Wellington,* [3]*Centre for Biodiversity and Restoration Ecology, School of Biological Sciences, Victoria University of Wellington,* [4]*Sydney Institute for Infectious Diseases, School of Life and Environmental Sciences and School of Medical Sciences, The University of Sydney, Australia.* [5]*Institute of Environmental Science and Research*	41
ID32	A Novel Rubi-Like Virus in the Pacific Electric Ray (*Tetronarce californica*) Reveals the Complex Evolutionary History of the *Matonaviridae*	Grimwood, R. G.[1], Holmes, E. C.[2], Geoghegan, J. L.[1,3].	[1]*Department of Microbiology and Immunology, University of Otago,* [2]*Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Life and Environmental Sciences and School of Medical Sciences, University of Sydney, Australia,* [3]*Institute of Environmental Science and Research*	42
ID33	Understanding the Innate Immune Response to Group A Streptococcus Pili	Risa Takahashi[1], Catherine Jia-Yun Tsai[2], Thomas Proft[2] and Nicole J Moreland[2]	[1]*Department of Molecular Medicine & Pathology, University of Auckland,* [2]*Department of Molecular Medicine & Pathology and Maurice Wilkins Centre*	43

ID34	How does antibody constant region diversity influence the immune response?	Warrender, A. K. [1], Pan, J. [1], Pudney, C. R.[2], & Kelton, W.[1]	[1]*Te Huataki Waiora School of Health, University of Waikato* [2]*Department of Biology & Biochemistry, University of Bath, UK*	44
ID35	Drug resistance is associated with collateral drug phenotypes in *Mycobacterium tuberculosis*	Waller, N.[1], Cheung, C.[1], McNeil, M.[1], Cook. G.[1]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin, NZ.*	45
ID36	Targeting the Cytochrome-bd Terminal Oxidase of *Mycobacterium tuberculosis*	Chen-Yi Cheung[1] and Gregory M. Cook[1,2]	[1] *Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand.* [2] *Maurice Wilkins Centre for Molecular Biodiscovery*	46
ID37	Gauging the exposure of bacteria to toxic oxidants inside neutrophil phagosomes	Nina Dickerhof [1], Heather A Parker[1], Mark B Hampton[1], Anthony J Kettle[1]	[1]*Centre for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago, Christchurch, NZ*	47
ID38	Sensitising *Streptococcus pneumoniae* to oxidative stress	Shearer, H.L.[1], Parker, H.A.[1], Pace, P.E.[1], Hampton, M.B.[1], Dickerhof, N.[1]	[1]*Centre for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago Christchurch, NZ*	48
ID39	The regulation of mucosal-associated invariant T (MAIT) cell activation by bacteria	Laura Wedlock[1], Rajesh Lamichhane[1], Sara M de la Harpe[2], Andrea J Vernall[2], James E Ussher[1,3]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin, NZ* [2]*School of Pharmacy, University of Otago, Dunedin, NZ* [3]*Southern Community Laboratories, Dunedin, NZ*	49
ID40	Bacterial resistance to oxidants in neutrophil phagosomes	Ashby, L.V., Dickerhof, N., Springer, R., Hampton, M.B., Kettle, A.J.	*Centre for Free Radical Research, Department of Pathology & Biomedical Science, University of Otago Christchurch, New Zealand.*	50
ID41	Exploiting synergistic interactions in energy metabolism to combat drug resistant *Mycobacterium tuberculosis*	Adolph C.R. [1,2], Cheung C.Y. [1], McNeil M.B. [1,2], and Cook G.M. [1,2]	[1] *Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, Dunedin NZ* [2] *Maurice Wilkins Centre for Molecular Biodiscovery*	51
ID42	Mathematical modelling of Covid-19 in New Zealand	Plank, M	*University of Canterbury, Christchurch New Zealand*	52
ID43	Understanding Natural Immunity to Streptococcus A Infections using validated Opsonophagocytic killing assays.	Reuben McGregor, Aimee Paterson, Lauren Carlton, Tiffany Chen, Nicole J Moreland	*Department of Molecular Medicine, University of Auckland, Auckland, New Zealand Maurice Wilkins Centre, Auckland, New Zealand*	53
ID44	Elimination and Beyond: A SARS-CoV-2 Serosurvey of Blood Donors in New Zealand	Lauren H. Carlton[1], Tiffany Chen[1], Alana L. Whitcombe[1],	[1]*School of Medical Sciences, The University of Auckland, Auckland, New Zealand*	54

		Reuben McGregor[1], Greg Scheurich[2], Campbell R. Sheen[3], James M. Dickson[4], Chris Bullen[5], Annie Chiang[5], Daniel J. Exeter[5], Janine Paynter[5], Michael G. Baker[6], Richard Charlewood[2], Nicole J. Moreland[1]	*[2]The New Zealand Blood Service, Auckland, New Zealand [3]Callaghan Innovation, Christchurch, New Zealand [4]School of Biological Sciences, The University of Auckland, NZ [5]School of Population Health, The University of Auckland, Auckland, New Zealand [6] Department of Public Health, The University of Otago, Wellington, New Zealand*	
ID45	Characterisation of carbapenem-hydrolysing β-lactamases for KIE based Transition-State analysis	Ruiz-Vargas, J.A.[1], Mittelstädt, G.[1], Parker, E.J.[1]	*[1]Ferrier Research Institute, Victoria University of Wellington, NZ.*	55
ID46	Induction of anti-SARS-CoV-2 polyclonal antibodies with cross-variant neutralising potency in ruminant milk	Jacobson, G.[1], Kraakman, K.[2,3], Wallace, O.[3], Pan, J.[1], Hennebry, A.[3], Smolenski, G.[3], Cursons, R.[1], Hodgkinson, S.[3], Williamson, A.[1], Kelton, W.[2]	*[1]Te Aka Mātuatua School of Science, University of Waikato, Hamilton, NZ, [2]Te Huataki Waiora School of Health, University of Waikato, Hamilton, NZ, [3]Ruakura Technologies Ltd, Ruakura, Ruakura Research Centre, Hamilton, NZ.*	56
ID47	Co-stimulatory function of 4-1BB during MAIT cell activation by bacteria	Lamichhane R, Williams J, Fouille R, Tirand C, Wedlock L, Hannaway R, Ussher JE	*Department of Microbiology and Immunology, University of Otago, Dunedin, NZ*	57
ID48	Phage-derived antimicrobials against kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*	Sisson, H.M.J.[1], Warring, S.L.[1], Dams, D.[2], Grimon, D.[2], Fagerlund, R.D.[1, 3], Gutiérrez, D.[2], Jackson, S.A.[1], Briers, Y.[2], Fineran, P.C.[1, 3]	*[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ. [2]Department of Biotechnology, Ghent University, Gent, Belgium. [3]Bioprotection Aotearoa, University of Otago, Dunedin, NZ.*	58
ID49	Mapping interactions between Group A Streptococcus (GAS) M-protein and antibodies	Prachi Sharma[1], Aimee Patterson[1], Reuben McGregor[1,2], Nicole Moreland[1,2]	*[1]School of Medical Sciences, the University of Auckland, New Zealand [2]Maurice Wilkins Centre, the University of Auckland, New Zealand*	59
ID50	Characterising the Immune Response in Acute Rheumatic Fever following Hydroxychloroquine Treatment	Francis Middleton[1], Reuben McGregor[1, 2], Nigel Wilson[3], Nicole J. Moreland[1, 2].	*[1]School of Medical Sciences, The University of Auckland, Auckland, New Zealand [2]Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand [3]Starship Children's Hospital, Auckland, New Zealand*	60

ID51	Disrupting Bacterial Metal Ion Homeostasis to Break Antimicrobial Resistance	Macbeth, E[1] ., Harbison-Price, N[1,2]., Ferguson, S.A[1] ., Cook, G.M[1,3]	*1 Department of Microbiology and Immunology, University of Otago, Dunedin, NZ* *2 School of Chemistry and Molecular Biosciences, The University of Queensland, Australia* *3 Maurice Wilkins Centre for Molecular Biodiscovery*	61
ID52	Investigation of antimicrobial tolerance in *Enterococcus faecalis*	Smith, M.J.B.[1], Cheung, C.Y. [1], Cook, G.M.[1], Darnell, R.L.[1],	*[1]Department of Microbiology and Immunology, University of Otago, Dunedin, NZ.*	62
ID53	The anti-viral role of host class II histone deacetylases in influenza A virus infection	Bennett Henzeler Esakialraj L[1], Ngoni Fay Husain M.[1,]	*Department of Microbiology and mmunology, University of Otago, Dunedin, New Zealand* *Genomics Aotearoa, Department of Biochemistry, University of Otago, Dunedin, New Zealand*	63
ID54	Multiplex transcriptional repression to investigate genetic interactions between bioenergetic complexes in *Mycobacterium tuberculosis*	Chapman, C.L[1] ., McNeil, M.B[1]., Cook, G.M[1]	*[1] Department of Microbiology and Immunology, University of Otago, Dunedin, NZ*	64
ID55	The Effect of Needle Length and Arm Size in mRNA COVID-19 Immunization	Paterson, A.[1], Hills, T.[2,3], Woodward, R.[4], Middleton, F.[1], Carlton, L.H.[1,] McGregor, R.[1], Barfoot, S.[1], Ramiah, C.[1], Whitcombe, A.L.[1], Morpeth, S.[5], Beasley, R.[2], Weatherall, M.[6,7], Jordan, A.[3], McIntyre, P.[7], Moreland, N.J.[1], Mirjalili, S.A.[1], and the "Hitting the Spot" Working Team[1]	*[1]School of Medical Sciences, The University of Auckland, New Zealand,* *[2]Medical Research Institute of New Zealand, New Zealand,* *[3]Auckland District Health Board, New Zealand,* *[4]Auckland Radiology Group Auckland Radiology Group, Auckland, New Zealand,* *[5]Counties Manukau District Health Board, New Zealand,* *[6]Capital and Coast District Health Board, New Zealand,* *[7]University of Otago Wellington, New Zealand*	65
ID56	Elevated IgG3 in Acute Rheumatic Fever	Ramiah, C.[1], Lorenz, N.[1], Sheen, C.[2], Sharma, P.[1], McGregor, R.[1], Moreland, N.J.[1]	*[1]Faculty of Medical Sciences, The University of Auckland, Auckland, New Zealand* *[2]Callaghan Innovation, Christchurch, New Zealand*	66
ID57	The molecular clock contributes to the host response to infection through regulating the complement system.	Rolland, L.[1], Du, L. [1], Keerthisinghe, P. [1], Hall, C.J.[1]	*[1]Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ*	67

ID58	The interplay between GAS virulence factors and a functional antibody response.	Lovell, J.R.[1], Paterson, A.[1], Loh, J.[1,2], Moreland, N.J.[1,2], McGregor, R.[1,2]	[1]*Faculty of Medical Sciences, University of Auckland.* [2]*Maurice Wilkins Centre, the University of Auckland, New Zealand*	68
ID59	Investigating the functional role respiratory oxidases play in the bacterial pathogen *Enterococcus faecalis*	Olivia Paxie[1], Francesca Todd Rose[1], James Cheung[1], Gregory Cook[1, 2] and Rachel Darnell[1]	[1]*Microbiology and Immunology Department, University of Otago* [2]*Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland*	69
ID60	Modelling Covid-19 like disease Spread Using Real Transport Flow	Reju Sam John[1] , Renata L. Muylaert[1] , Joel C. Miller[2] , David TS Hayman[1]	[1:] *mEpiLab, Hopkirk Research Institute, School of Veterinary Science, Massey University, New Zealand* [2] *: Department of Mathematical and Physical Sciences, La Trobe University, Melbourne, Australia*	70
ID61	Feasibility of using a luminescence-based method to determine serum bactericidal activity against *Neisseria gonorrhoeae*	Fiona Clow[1], Conor J O'Hanlon[1], Myron Christodoulides[2], Fiona J Radcliff[1]	[1]*University of Auckland, New Zealand* [2]*University of Southampton, United Kingdom.*	71
ID62	Metabolic Dysregulation in Drug-resistant *Mycobacterium tuberculosis*	Wang, XY.[1], Cheung, J.[1], Jackson, S.[1], Cook, G.M.[1], McNeil, M.[1]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin, NZ,*	72
ID63	Can BCG vaccination train innate immune cells to protect against skin and soft tissue infections?	Sircombe, K.J.[1], Kirman, J.R.[1], Pletzer, D.[1]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin, NZ.*	73
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ID65	Exploiting metabolic dysregulation to potentiate antibiotic killing of resistant *Mycobacterium tuberculosis*	Jowsey W.J.[1,2], McNeil M.B.[1,2], Cook G.M.[1,2]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand* [2]*Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland, New Zealand*	75
ID66	Development of a COVID-19 rapid antigen test using filamentous phage-derived nanorods as detector particles	Davenport, C.[1,2,3], Le, V.V.H.[1,3], Gonzalez-Miro, M.[1], Rakonjac, J.[1,2,3]	[1]*School of Natural Sciences, Massey University, Palmerston North, NZ.* [2]*Nanophage Technologies.* [3]*Maurice Wilkins Centre.*	76
ID67	Using antibiotic combinations to suppress development of resistance	Lyons, N.M.A.[1,2], Wu, W.[3], Jin, Y.[3], Lamont, I.L.[1], Pletzer, D.[2]	[1]*Department of Biochemistry, University of Otago, Dunedin, NZ,* [2]*Department of Microbiology & Immunology, University of Otago, Dunedin, NZ,*	77

			[3]*Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, China.*	
ID68	Post infection cellular and humoral immune responses of New Zealanders to SARS-COV-2	Elham Torbati[1], Alyson Craigie[1], Susan Jack[2], Arlo Upton[3], and James Ussher[1,3]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin* [2]*Public Health South, Southern District Health Board, Dunedin* [3]*Southern Community Laboratories, Dunedin*	78
ID69	Serine acetyltransferase from *Neisseria gonorrhoeae*; structural and biochemical basis of inhibition	Oldham, K. E.,[1,2] Prentice, E. J., Summers, E. L., & Hicks, J. L.[1]	[1]*School of Health, University of Waikato, Hamilton, NZ,* [2]*School of Science, University of Waikato, Hamilton, NZ.*	79
ID70	Investigation of a causative agent for Respiratory Distress Syndrome in neonatal hoiho	Wierenga, J.R.[1,2], Morgan, K.J.[2], Hunter S[2], Taylor H[3], McInnes K[3], Geoghegan, J.L.[1]	[1]*Department of Microbiology and Immunology, University of Otago, Dunedin, NZ,* [2]*School of Veterinary Science, Massey University, Palmerston North, NZ,* [3] *Biodiversity Group, Department of Conservation Te Papa Atawhai, Wellington, NZ*	80
ID71	Structural and biochemical characterisation of *O*-acetylserine sulphydrylase (CysK) from *Neisseria gonorrhoeae*	McGarvie, J.[1] & Hicks, J.[2]	[1]School of Science, University of Waikato, Hamilton, NZ [2] Te Huataki Waiora, School of Health, University of Waikato, Hamilton, NZ.	81
ID72	Towards a New Class of Anti-Tuberculosis Drugs: Design and Synthesis of AnPRT Transition State Analogues	Mason, E.J.; Lamiable-Oulaidi, F.; Parker, E.; Tyler, P.	Ferrier Research Institute, Victoria University of Wellington, NZ.	82
ID73	Understanding and targeting tryptophan biosynthesis in human pathogens	Scully, T. & Parker, E.	Ferrier Research Institute, Victoria University of Wellington, NZ.	83
ID74	High-resolution mapping of IgA receptor interactions by deep mutational scanning	Hanning, K.R[1]., Pan, J.[2] & Kelton, W[1].	[1]School of Health, University of Waikato, Hamilton, NZ [2] School of Science, University of Waikato, Hamilton, NZ.	84

ID27: A Novel Recombinant *Lactococcus lactis* Mucosal Vaccine Platform Based on Group A Streptococcus Pili

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Well-defined synthetic peptides are attractive for vaccine development. However, they are usually poorly immunogenic and sensitive to proteolytic degradation, thus require conjugation to carrier proteins and/or addition of adjuvants. Lactic acid bacteria (LAB) have become promising vehicle for mucosal vaccines due to their safety profile and natural adjuvanticity. The choice of carrier and mode of presentation hugely affect the stability and immunogenicity of the antigen, thus determine effectiveness of the resulting vaccines. We propose that the group A streptococcus (GAS) pilus structure expressed on the surface of *Lactococcus lactis* can be an ideal carrier for antigenic peptides, and established a novel mucosal vaccine platform termed PilVax[1]. Pili (*sing.* pilus) are hair-like bacterial cell surface protrusions important for host cell adhesion. The GAS pili consist of covalently linked pilins that are structurally stable and highly immunogenic. We identified several regions within the backbone pilin that can be replaced with antigenic peptides. Expressing the peptides within the pilus structure allows for peptide amplification, stabilisation and enhanced immunogenicity. Intranasal immunisation of mice with the resulting recombinant *L. lactis* strain produced strong peptide-specific antibody responses in serum and bronchoalveolar fluid. A recently developed tuberculosis vaccine based on a dominant T-cell epitope generated both humoral and cellular immune responses in the immunised mice[2]. PilVax vaccination resulted in peptide-specific $CD4^+$ T cells at levels similar to those resulting from BCG immunisation, as well as an unexpected increase in the numbers of $CD3^+CD4^-CD8^-$ (double negative [DN]) T cells in the lungs of vaccinated animals. These cells types were shown to be responsible for the cytokine production following stimulation with the cognate peptide. These results demonstrate the suitability of developing PilVax into useful mucosal vaccines.

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ID28: Bacterial competence or biofilm formation? Potential roles of the gonococcal periplasmic ligase, Lig E.

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The rapid rise in multi-drug resistant *Neisseria gonorrhoeae* isolates has caused a growing public concern on the treatment of its sexually transmitted disease, gonorrhoea. Although this bacterium affects both men and women, symptoms can vary greatly. Previously easily treatable, gonorrhoea is now spreading at alarming rates, due to the bacterium's unregulated ability to take up environmental DNA. With approximately 106 million new cases annually, thorough investigations into novel gonococcal pathways that can be targeted are crucial.[1]

In particular, disruption of the DNA repair system would greatly discourage bacterial growth. This includes ligases that are important at sealing DNA breaks. In particular, *N. gonorrhoeae* expresses a minimal ATP-dependent ligase, Lig E, which contains a signal peptide that indicates a likely periplasmic location, as opposed to the cytoplasmic location of DNA.[2] Thus, we hypothesise that Lig E has a role in bacterial competence and aids in repairing damaged environmental DNA, thus enhancing the acquisition of antibiotic resistance genes in *N. gonorrhoeae* under DNA damaging conditions.

To identify the importance of Lig E from *N. gonorrhoeae* (Ngo-Lig) on the bacterium's growth, we have generated *in vivo* mutants of Ngo-Lig, the effects of which were characterised *in vivo* (growth experiment) and *in vitro* (ligation assays). Results from these demonstrated a decrease in the rates of gonococcal growth when *Lig E* is disrupted, although its growth trajectory still follows a characteristic bacterial growth curve. Thus, we believe that in addition to its role in DNA uptake, Lig E may also be important for biofilm formation, which is critical for gonococcal attachment and infection of human cells. Although further research into this is necessary, the results collected demonstrated a novel pathway that may be targeted by future drug developments to tackle this emerging threat in our community.

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ID29: Developing an mRNA vaccine for malaria

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Malaria is a debilitating and deadly disease caused by *Plasmodium* parasites, transmitted by *Anopheles* mosquitoes in tropical regions. In 2019, the WHO estimated there were over 200 million active infections, resulting in 400,000 deaths a year. Following a blood meal, sporozoites from an infected mosquito travel to the liver where they infect hepatocytes, reproduce and develop into the merozoite stage of their life cycle, which ultimately break-out of the liver and infect red blood cells (RBCs). Merozoites continue to reproduce asexually and infect RBCs, causing anaemia, organ failure and death. Current treatments include Artemisinin Combination Therapy (ACT), which is used in combination with other anti-parasitic drugs to increase efficacy against drug-resistant strains. The only current vaccine is an AS01-adjuvanted virus-like particle that generates antibodies against the well-characterised malaria sporozoite antigen, circumsporozoite protein (CSP) to prevent infection of the liver. This vaccine is suboptimal providing ≤30% protection after 1 year and this efficacy decreases over time. Recently, a type of $CD8^+$ T cell that resides in the liver, named liver tissue-**r**esident **m**emory **T** cells (liver T_{RM}) have been shown to provide protection from malaria by killing sporozoite-infected hepatocytes and halting the malaria life cycle before reaching the pathogenic blood-stage. Here we will report the development of a mRNA vaccine formulation incorporating novel NKT cell agonists, mRNA coding for malaria antigen and a lipid delivery system that can generate robust liver T_{RM} cell responses that can protect against malaria sporozoite challenge.

ID30: Movement for Change: Structural Rearrangement and Interaction of the Transcriptional Regulator CysB with DNA

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted infection gonorrhoea has become resistant to every antibiotic used for its treatment, earning it 'superbug' status. Coupled with high asymptomatic carriage means we are at risk of not being able to treat this disease in the future. Gene expression and regulation are at the heart of *N. gonorrhoeae's* success as a pathogen and its ability to adapt to the host environment during infection. *N. gonorrhoeae* has ~34 transcriptional regulators compared to other bacteria such as *Escherichia coli* which have greater than 200.
These few transcription regulators found in *N. gonorrhoeae* include CysB which is differentially expressed upon infection[1]. CysB plays a crucial role in regulating sulphur metabolism and cysteine synthesis. However, little is known about the role of CysB during infection, and the structural rearrangements that occur within the protein upon ligand and DNA binding. We have solved the first full-length structure of CysB by X-ray crystallography, and are using SAXS to investigate conformational changes upon DNA and ligand binding giving us unique insight into regulation of gene expression by structural rearrangements in CysB. We are also characterising CysB DNA binding *in vitro* by assaying binding to specific cysteine regulon promoters in the presence and absence of inducer. Our inability to delete *cysB* from the *N. gonorrhoeae* chromosome supports the essentiality of this gene[2]. Understanding the biology of *N. gonorrhoeae* and how it senses and responds to the environment at the DNA level is key for the development of new strategies to prevent gonorrhoea infection.

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ID31: Cloacal virome of an ancient host lineage – the tuatara (*Sphenodon punctatus*) – reveals abundant and diverse diet-related viruses

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Tuatara (*Sphenodon punctatus*) are one of the most phylogenetically isolated species and provide a unique host system to study virus evolution. While the tuatara genome, sequenced in 2020, revealed many endogenous viral elements, we know little of the exogenous viruses that infect tuatara. We performed a metatranscriptomics study of tuatara cloaca samples from a wild population on Takapourewa (Stephens Island), Aotearoa New Zealand. From these data we identified 49 potentially novel viral species that spanned 20 RNA viral families and/or orders, the vast majority (48) of which were likely dietary related. Notably, using a protein structure homology search, we identified a highly divergent novel virus within the *Picornaviridae* which may directly infect tuatara. Additionally, two endogenous tuatara adintoviruses were characterised that exhibited long-term viral-host co-divergence. Overall, our results indicate that the tuatara cloacal virome is highly diverse likely due a large number of dietary related viruses.

ID32: A Novel Rubi-Like Virus in the Pacific Electric Ray (*Tetronarce californica*) Reveals the Complex Evolutionary History of the *Matonaviridae*

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Rubella virus (RuV) is the causative agent of rubella ("German measles") and remains a global health concern. Until recently, RuV was the only known member of the genus *Rubivirus* and the only virus species classified within the *Matonaviridae* family of positive-sense RNA viruses. Recently, two new rubella-like matonaviruses, *Rustrela virus* and *Ruhugu virus*, have been identified in several mammalian species, along with more divergent viruses in fish and reptiles. To screen for the presence of additional novel rubella-like viruses, we mined published transcriptome data using genome sequences from *Rubella*, *Rustrela*, and *Ruhugu* viruses as baits. From this, we identified a novel rubella-like virus in a transcriptome of *Tetronarce californica* – order Torpediniformes (Pacific electric ray) – that is more closely related to mammalian *Rustrela virus* than to the divergent fish matonavirus and indicative of a complex pattern of cross-species virus transmission. Analysis of host reads confirmed that the sample analysed was indeed from a Pacific electric ray, and two other viruses identified in this animal, from the *Arenaviridae* and *Reoviridae,* grouped with other fish viruses. These findings indicate that the evolutionary history of the *Matonaviridae* is more complex than previously thought and highlights the vast number of viruses that remain undiscovered.

ID33: Understanding the Innate Immune Response to Group A Streptococcus Pili

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Despite Group A Streptococcus infections and complications imposing a global disease burden, there is no available vaccine against this pathogen. One potential vaccine candidate is the GAS pilus, a long, hair-like structure expressed on the cell surface and key for the initiation of infection. Pili based vaccines have the potential to provide broader strain coverage compared to the more extensively studied candidates such as M protein. They have been demonstrated to stimulate robust production of protective antibodies, but the innate immune responses involved remain undefined.

Pilus recombinant proteins and recombinant *L. lactis* strains expressing GAS pili on the cell surface were utilised to investigate the immunomodulation capacity of this surface structure. Interactions between pili and toll-like receptors (TLRs) was studied in HEK239 reporter cell lines expressing various human TLRs. Cytokine production in response to pili was analysed using ELISA in the monocyticTHP-1 cells.

Protein production downstream of TLRs in the HEK239 cell lines indicated specificity of pili for TLR2, and the interaction between TLR2 and pili subunits was confirmed via binding assays. Furthermore, the TLR2/6 heterodimer was pinpointed as the TLR2 heterodimer recognising pili. In the THP-1 cells, strong pili induced production of pro-inflammatory cytokines such as TNFα was observed. Whilst both the tip and backbone subunit appeared to be involved in the innate immune response, the tip subunit was found to have higher affinity binding to receptor and induced higher levels of cell stimulation. Interestingly, differences in the levels of cellular response were seen between different GAS pilus types.

Insight into the immunomodulatory characteristics of the GAS pilus will assist in determining the structure's ability to achieve a desirable immunisation outcome. This will help ascertain the prospects of the pili as a GAS vaccine candidate, as well as aid assessment of its potential as an adjuvant in other vaccines

ID34: How does antibody constant region diversity influence the immune response?

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Antibodies are central to frontline immune defence against infection by detecting invading pathogens and activating immune cells to neutralise or kill them. Precise pathogen targeting is governed by highly adaptable antibody variable regions that confer high-affinity binding with fine epitope specificity. While the constant region is typically responsible for binding to Fc receptors on immune cells, evidence is emerging that it may influence the specificity and affinity of antigen binding also. With over 250 naturally occurring antibody constant region alleles discovered over the past four years, the functional consequence of a large proportion of this unexpected diversity remains unknown.

To study these unique antibody variants in fine detail, we have expressed and purified a panel of 35 alleles with non-synonymous mutations from subclasses IgG1, IgG2, and IgG3. We used high-resolution Liquid Chromatography-Mass Spectrometry to characterise glycan profiles for each variant. Despite heterogeneous profiles being resolved, the G0 F glycoform emerged as most dominant for all variants, in line with previous reports for clinical antibodies. This analysis allows deconvolution of the role of posttranslational modification versus amino acid changes on function. Subsequently, a stability analysis using a technique called Red Edge Excitation Shift spectroscopy was undertaken to first understand whether these alleles are likely a product of natural mutational drift or whether alternate selection pressures (i.e. altered receptor binding) have shaped the observed diversity. Our analysis has found differences in allelic stability and provides a rationale for testing individual receptor binding kinetics that shape adaptive immune responses in future.

ID35: Drug resistance is associated with collateral drug phenotypes in *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is the causative agent of tuberculosis, a disease that kills approximately 1.3 million people each year [1]. Currently a curable disease, treatment requires a multi-drug regimen of four drugs for 6-9 months. However, treatment of drug-resistant (DR) *M. tuberculosis* strains is becoming increasingly difficult, with most therapies showing only a 0-50% treatment success rate [2]. Novel treatment strategies to rapidly eliminate and prevent the emergence of drug resistance are needed. Drug resistance in *M. tuberculosis* is driven by chromosomal mutations that alter the structure or expression of antibiotic targets or resistance genes. Many of these genes play crucial roles in central cellular processes including cell wall synthesis, metabolism, transcription, and translation. I hypothesize that mutations responsible for antimicrobial resistance in *M. tuberculosis* will have secondary 'collateral' impacts, resulting in increased sensitivity to drug inhibition.

To investigate the collateral impacts of drug resistance in *M. tuberculosis* I have generated a collection of mono-resistant strains in a PC2-approved avirulent background of *M. tuberculosis*. Whole-genome sequencing and antimicrobial susceptibility profiling of resistant strains against a panel of diverse antibiotics was performed to identify examples of increased antibiotic susceptibility. These results demonstrated that diverse clinically relevant resistance mechanisms have both unique and overlapping collateral increases in antibiotic susceptibility. For example, mutations in the *katG* catalase increase sensitivity to all of Q203, PA824, and the ATP synthase inhibitor BDQ. Here, I will expand upon these results to highlight how the collateral impacts of drug resistance increases susceptibility and killing by alternative antibiotics.

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ID36: Targeting the Cytochrome-bd Terminal Oxidase of *Mycobacterium tuberculosis*

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Increasing antimicrobial resistance compels the search for next-generation inhibitors with differing or multiple molecular targets. In this regard, energy conservation in *Mycobacterium tuberculosis* has been clinically validated as a promising new drug target for combatting drug-resistant strains of *M. tuberculosis,* and of particular note are the terminal oxidases*. M. tuberculosis* shows a high level of plasticity in utilizing both terminal oxidases for growth and survival and inhibition of both terminal respiratory oxidases simultaneous is required for bactericidal activity[1]. Q203 has been developed as an inhibitor of *M. tuberculosis* cytochrome bcc:aa_3 terminal oxidase, one of two terminal oxidases that catalyze the terminal reduction of oxygen during cellular respiration. Although bacteriostatic on its own, Q203 is rapidly and potently bactericidal when a secondary terminal oxidase, cytochrome bd, is deleted[2]. We propose that cytochrome bd represent a promising target for antitubercular drug development, particularly in combination with Q203[3].

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ID37: Gauging the exposure of bacteria to toxic oxidants inside neutrophil phagosomes

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Neutrophils ingest bacteria into intracellular compartments called phagosomes, and subject them to a cocktail of antimicrobial peptides and proteins, and oxidants. Myeloperoxidase is considered a key player in this environment as it converts chloride to hypochlorous acid (HOCl) - a potent bactericidal oxidant. However, whether enough HOCl is produced inside neutrophil phagosomes to be responsible for killing ingested bacteria has been a matter of ongoing debate. To gauge the exposure of different bacteria to HOCl inside the phagosome, we monitored oxidation of bacterial low molecular weight thiols (LMWTs) in *Staphylococcus aureus, Mycobacterium smegmatis* and *Pseudomonas aeruginosa*. These bacteria were treated with reagent HOCl and their major LMWT (glutathione, bacillithiol or mycothiol) was measured by liquid chromatography with mass spectrometry. LMWTs were oxidized upon treatment with increasing doses of HOCl in all three bacteria, which was mirrored by microbial death. The oxidation pattern of bacterial LMWTs during neutrophil phagocytosis indicated that HOCl was sufficient to be solely responsible for killing of *P. aeruginosa*, but only partially contributed to killing of *S. aureus*. Mycothiol was not oxidized in phagosomal *M. smegmatis*, suggesting that these bacteria are able to resist the doses of HOCl generated in the phagosome. Our investigations demonstrate that depending on the type of bacteria ingested by neutrophils, oxidants may be solely responsible for killing, work alongside other toxins, or contribute little to eradication of the bacteria. Sensitizing *S. aureus* and mycobacteria to phagosomal HOCl may assist neutrophils in killing these bacteria.

ID38: Sensitising *Streptococcus pneumoniae* to oxidative stress

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Streptococcus pneumoniae is a commensal bacterium that colonises mucosal surfaces in the upper respiratory tract in humans and is the leading cause of pneumonia. Interestingly, this bacterium generates hydrogen peroxide (H_2O_2) as part of its metabolism but cannot degrade it. We contend that in the human respiratory tract, this H_2O_2 will be utilised by host peroxidase enzymes to form the oxidants hypochlorous acid (HOCl) and hypothiocyanous acid (HOSCN). Whether like H_2O_2, *S. pneumoniae* can tolerate HOCl and HOSCN was previously unknown. We examined the susceptibility of this bacterium to H_2O_2, HOCl and HOSCN by assessing bacterial viability following oxidant exposure using colony forming unit assays. The most striking difference identified was that *S. pneumoniae* was considerably more tolerant to HOSCN than *Pseudomonas aeruginosa*, another respiratory tract pathogen. We investigated the importance of *S. pneumoniae*'s main low-molecular-weight thiol glutathione for protecting against HOSCN. When the genes *gshT*, encoding a substrate binding protein required for glutathione import, or *gor*, encoding the enzyme that recycles oxidised glutathione, were knocked out, S*. pneumoniae* tolerance to HOSCN was decreased. We also identified a previously uncharacterised HOSCN reductase enzyme in *S. pneumoniae*. We found that this enzyme contributes to HOSCN tolerance, as when it was knocked out alongside the glutathione antioxidant system, no bacterial growth occurred in a HOSCN generating system. In summary, this work has identified novel targets to sensitise *S. pneumoniae* to the immune-derived oxidant HOSCN. Targeting the ability of *S. pneumoniae* to tolerate HOSCN may be a viable approach for combating this deadly pathogen.

ID39: The regulation of mucosal-associated invariant T (MAIT) cell activation by bacteria

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Mucosal associated invariant T (MAIT) cells are abundant innate-like T cells in humans that are characterised by the expression of a semi-invariant T cell receptor (TCR), Vα7.2-Jα33/12/20, that recognizes bacteria-derived pyrimidine derivatives from bacterial riboflavin biosynthesis, such as 5-(2-oxyopropylideneamino)-6-D-amino-ribityluracil (5-OP-RU), presented by MR1 (a MHC-I like molecule). Upon early activation by riboflavin-producing bacteria such as *Escherichia coli*, MAIT cells robustly upregulate cell surface proteins (e.g. CD69) and produce pro-inflammatory and cytotoxic molecules, and this response is dependent on MR1-TCR interaction. However, it is unclear which other bacterial signals regulate early MAIT cell activation.

In this study, the role of bacterial signals during early MAIT cell activation by 5-OP-RU was investigated *in vitro* using a riboflavin-deficient *E. coli* mutant (*E. coli* Δ*ribD*), and various agonists and antagonists of innate signalling pathways. We observed that presence of ligand-deficient *E. coli* Δ*ribD* resulted in enhanced MAIT cell activation in response to 5-OP-RU which was predominantly MR1 mediated. Transcriptomic analysis demonstrated that several innate signaling pathways were enriched in MAIT cells stimulated by *E. coli*. Consistently, cytokines such as IL-2, IL-7, and type I interferons (T1-IFNs) upregulated MAIT cell activation by 5-OP-RU. Blocking T1-IFNs significantly reduced MAIT cell activation by *E. coli* HB101 and abrogated the enhanced MAIT cell response to 5-OP-RU in the presence of *E. coli* Δ*ribD* or the agonists of toll-like receptors 1/2 and 4. Therefore, various innate signals including T1-IFNs co-operate with MR1 ligands to modulate MAIT cell activation.

ID40: Bacterial resistance to oxidants in neutrophil phagosomes

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Neutrophils clear infections by ingesting bacteria into phagosomes and then killing them with oxidants and antimicrobial proteins. Some pathogenic bacteria, however, survive inside phagosomes and seed serious infections. We need to better understand the killing mechanisms used inside phagosomes to develop new strategies to combat persistent bacteria. Our focus is on oxidative killing. Recently, we demonstrated considerable heterogeneity in oxidant production within phagosomes. Now, we are investigating the interplay between toxic hypohalous acids produced by the enzyme myeloperoxidase and the microbes trapped in phagosomes. Bacteria protect themselves with myriad defence mechanisms. These include thiol-rich molecules to act as an antioxidant sink, such as bacillithiol, a low molecular weight thiol that is important for bacterial virulence. We have a mutant strain of *Staphylococcus aureus* lacking bacillithiol to compare with the wildtype strain. Our studies also address the neutrophil's production of reactive oxidants in the phagosome, and the modulation of toxic products by myeloperoxidase. We have developed assays using freshly isolated human neutrophils to study the killing of bacteria inside phagosomes over short and extended time courses. We show that *S. aureus* lacking bacillithiol are slightly more sensitive to neutrophil killing in our system compared to the wildtype strain. The increase in susceptibility was relatively subtle, likely due to the multifactorial defences of bacteria as well as multiplicity of non-oxidative mechanisms employed by the neutrophil. We also show that phagosomal killing rates can be modified by altering the balance of halide substrates available to myeloperoxidase in its generation of hypohalous acids. Again, the differences in neutrophil killing were small but significant. Our findings point to a complex interplay between the toxins deployed against bacteria within phagosomes and the strategies they use to combat them. We conclude that bacteria replete with antioxidant defences may survive in phagosomes that have relatively low oxidant production.

ID41: Exploiting synergistic interactions in energy metabolism to combat drug resistant *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a leading infectious cause of death worldwide, resulting in 10 million new cases and 1.5 million deaths in 2020. Treatment of infections with *Mycobacterium tuberculosis*, the primary causative agent of TB, is challenging and requires six months of combinational therapy. Infections with multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* are even more difficult to treat, requiring up to two years of chemotherapy with more toxic and less effective second-line agents, and have unacceptably high mortality rates. Thus, global TB control urgently depends on the development of new drugs and drug regimens that can quickly and effectively treat both drug-sensitive and drug-resistant TB infections.

Mycobacterial energy generation is an area showing promise for the development of new faster acting and highly potent drugs. Succinate dehydrogenase (SDH), or complex II of the electron transport chain, couples the two-electron oxidation of succinate, a TCA cycle metabolite, to the reduction of quinone (succinate + quinone ↔ fumarate + quinol), thereby directly linking central carbon metabolism to the respiratory chain. The dual role of SDH within both the TCA cycle and respiratory chain of *M. tuberculosis* makes it a particularly attractive drug target. However, to date no mycobacterial specific SDH inhibitors have been identified. Here, we address this by reporting on the identification of novel SDH inhibitors that inhibit the growth of *M. tuberculosis* at low micromolar concentrations. Moreover, we identify that chemical or genetic inhibition of other respiratory chain complexes is synergistic or synthetically lethal with SDH inhibition, suggesting that SDH inhibitors may be useful in multi-drug regimens.

ID42: Mathematical modelling of Covid-19 in New Zealand

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Since March 2020, Covid-19 Modelling Aotearoa (formerly part of Te Pūnaha Matatini) has been using mathematical models of the spread and impact of Covid-19 in New Zealand to support the government response. In this talk, I will present some of the ingredients that go into these models in non-technical terms. I will talk about what can (and can't) be learnt from models, how they have been used for policy advice, and some of the challenges and opportunities in this area.

ID43: Understanding Natural Immunity to Streptococcus A Infections using validated Opsonophagocytic killing assays.

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Background: Streptococcus A (StrepA) is a globally important pathogen for which there is no licenced vaccine. Understanding the natural, protective immune response to the pathogen will inform the development of an effective vaccine. However, the basis of population immunity to StrepA is poorly understood. The most widely used assays for assessing the protective capacity of sera are known as "Lancefield" bactericidal assays. These assays were developed in the 1950s and are not suitable for contemporary vaccine programmes, which require an assay that is quantitative, reproducible and high-throughput.

Methods: We have studied the natural antibody response developed to StrepA using pooled human intravenous immunoglobulin G (IVIG), combined with our newly developed StrepA opsonophagocytic killing assay (OPKA). The IVIG is purified from over one thousand healthy donors, and is therefore a meaningful surrogate of population wide immunity. We optimised the OPKA for StrepA strains that are representative of the three major StrepA pattern-types; M12 (A-C pattern), M53 (D pattern) and M75 (E pattern).

Results: While IVIG is capable of killing each of these StrepA strains, specificity assays showed the profile of protective antibodies differs for each strain. The M protein is a key virulence factor and the basis of leading StrepA vaccines. Interestingly, antibodies targeting M protein were a major component of the IVIG protective antibody repertoire for M12, intermediate for M53, but not for M75.

Conclusion: In summary, contemporary bactericidal assays provide a robust means to map the protective StrepA immune response, with notable differences in the contribution of M protein antibodies to opsonophagocytosis across the three major pattern types.

ID44: Elimination and Beyond: A SARS-CoV-2 Serosurvey of Blood Donors in New Zealand

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New Zealand had a strategy of eliminating SARS-CoV-2 that had resulted in a low incidence of reported coronavirus-19 disease (COVID-19) prior to the delta outbreak in August, 2021. The aim of this study was to describe the spread of SARS-CoV-2 in New Zealand via a nationwide serosurvey of blood donors. Samples (n=9806) were collected over a month-long period (December 3rd, 2020 - January 6th, 2021) from donors aged 16-88 years. The sample population was geographically spread, covering 16 of 20 district health board regions. A series of Spike-based immunoassays were utilized, and the serological testing algorithm was optimized for specificity given New Zealand was a low prevalence setting at the time. Samples were screened with a well-validated 2-step ELISA that comprises a single point dilution against the Spike receptor binding domain followed by titration against trimeric Spike protein. Samples above the cut-off were tested on two commercial immunoassays (EuroImmun SARS-CoV-2 IgG ELISA and cPass surrogate Viral Neutralization Test) and deemed seropositive if above the cut-off on both. Just eighteen samples were seropositive, of which six were retrospectively matched to previously confirmed COVID-19 cases. Four were from donors that travelled to settings with a high risk of SARS-CoV-2 exposure, suggesting likely infection outside New Zealand. The remaining eight seropositive samples gave a true seroprevalence estimate, adjusted for test sensitivity and specificity, of 0.103% (95% confidence interval, 0.09-0.12%). The very low seroprevalence is consistent with limited undetected community transmission at the time. Since then, New Zealand had a successful roll-out of a Spike-based mRNA vaccine and a large and ongoing omicron outbreak. This changes the design and purpose of future serosurveys, including the ability to inform population level immunity and waning over time. Temporal data from a limited number of blood donors will be presented to demonstrate this potential utility.
Word count: 298

ID45: Characterisation of carbapenem-hydrolysing β-lactamases for KIE based Transition-State analysis

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Antibiotic resistance continues to be a very concerning threat to worldwide human health. For decades, β-lactam antibiotics (e.g. penicillins, cephalosporins and carbapenems) have been the most frequently prescribed drugs for the treatment of most bacterial infections. However, their extensive use has led to the emergence of antibiotic-resistant bacteria capable of deactivating β-lactams, mainly through the action of enzymes called β-lactamases. These enzymes continue to evolve and spread around the world, putting our ability to treat bacterial infections with the most common antibiotics at great risk.

This project is focused on the carbapenemases (i.e. the β-lactamases able to hydrolyse all classes of β-lactams) that provide bacteria with multidrug resistance or "superbug" status.1 The most predominant enzymes of this category are the KPC-2, NDM-1 and OXA-48 β-lactamases. So far, we have heterologously expressed and purified these enzymes and investigated their activity using benzylpenicillin and meropenem as substrates. The solid understanding of the β-lactam hydrolysis reaction kinetics of these enzymes is being used as the basis to set up a Kinetic Isotope Effects (KIEs) methodology for accessing important details of their transition-states. Transition-state analysis has proven to be a powerful technique for the development of potent enzyme inhibitors, hence its implementation with the β-lactamases may facilitate the design and discovery of novel β-lactamase inhibitors capable of deactivating these menacing enzymes.

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ID46: Induction of anti-SARS-CoV-2 polyclonal antibodies with cross-variant neutralising potency in ruminant milk

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Immune-derived antibodies have become important tools for the treatment and detection of SARS-CoV-2 infections but the large-scale production of these biologics remains challenging. One promising avenue for the rapid and scalable generation of anti-SARS-CoV-2 polyclonal antibodies is the immunisation of lactating ruminant animals that facilitates the transfer of neutralising responses into hyperimmune milk/colostrum. Antigen-specific antibodies can then be extracted or formulated directly from these fractions. As well as a source of diagnostic reagents, hyperimmune milks with anti-SARS-CoV-2 bioactivity have potential as functional food supplements. However, the potency and magnitude of the antibody response generated by immunisation is critical to the quality of these preparations.

Here, we designed a series of ruminant-specific SARS-CoV-2 antigens to induce polyclonal antibodies in milk/colostrum. Our antigens were created by fusion of RBD or Spike proteins (Wuhan-Hu-1) to key protein elements of host immunity to enhance immunogenicity. In an ovine immunisation trial, we found significantly elevated levels of SARS-CoV-2-specific neutralising antibodies were generated by our fusion designs relative to unmodified antigens. Importantly, we observe transfer of these antibodies into milk/colostrum following lambing. Using surrogate viral neutralisation assays, we determined the cross-variant neutralising potency of the antibody fraction is retained for B. 1.351 (beta) and B.1.617.2 (delta) variants. Activity is also preserved to a lesser extent against the B.1.1.529 (omicron) variant in contrast to clinical antibodies REGN33/REGN87. In the future, our modular antigens can be redesigned to elicit antibody responses against other viral pathogens relevant to human and animal health.

ID47: Co-stimulatory function of 4-1BB during MAIT cell activation by bacteria

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Mucosal associated invariant T (MAIT) cells are abundant unconventional T cells in humans which can be stimulated in a T-cell receptor (TCR) dependent manner by a pyrimidine ligand derived from riboflavin synthesizing bacteria. The MAIT cell activating ligands were discovered to be the product formed after the reaction of 5-amino-6-D-ribitylaminouracil (5-A-RU), unstable pyrimidine intermediate of riboflavin pathway, with methylglyoxal (MG). Bacteria do not only provide the MAIT cell ligand but also trigger production of soluble cytokines or upregulation of co-stimulatory molecules on antigen presenting cells that can modulate TCR dependent activation of MAIT cells. Although previous studies reported some co-stimulatory signals from bacteria modulate MAIT cell activation both in *in vitro* and *in vivo*, the mechanisms by which MAIT cell activation is regulated during bacterial infection remain unclear.

TNF receptor family molecules are one of the important co-stimulatory molecules for T cell activation and are only recently getting attention in the context of MAIT cell activation. In this study, we activated MAIT cells by various TCR and non-TCR signals in the human peripheral blood mononuclear cells (PBMCs) system. Using flow cytometry, we showed that 4-1BB is the most highly expressed TNF co-stimulatory molecule on MAIT cells compared to Ox40 and CD30 during early bacteria mediated activation. Both 4-1BB and Ox40 are expressed at later time points infection. We also found marked differences in the expression of cell surface molecules, inflammatory cytokines, and transcription factors between the 4-1BB^{+} and 4-1BB^{-} MAIT cells suggesting MAIT cells expressing 4-1BB during activation are functionally superior. We further demonstrated increased 4-1BB ligation to its ligand in a co-culture system result in an enhanced activation and cytokine response by MAIT cells against early and late TCR dependent activation. Overall, our findings established an important role for 4-1BB expression during MAIT cell activation by bacteria.

ID48: Phage-derived antimicrobials against kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*

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Horticultural diseases caused by bacterial pathogens provide a consistent obstacle to crop production globally. The 2010 outbreak of the gram-negative kiwifruit phytopathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*) in New Zealand caused an estimated >$1 billion impact on the NZ economy[1]. Agrichemical sprays including copper and antibiotics have been used to manage *Psa* to date[2]. The rising levels of bacterial resistance requires the development of new antimicrobials, particularly specific, environmentally friendly, sustainable solutions. Bacteriophages – viruses that specifically infect and kill bacteria – are one option as biocontrol agents. An alternative phage-derived biocontrol strategy is the use of endolysins, lytic enzymes produced by phages at the end of their replication cycle. These enzymes lyse bacterial cells from the 'inside out' by degrading the peptidoglycan (PG) of the cell wall[3]. Exogenous application of lysins has shown antimicrobial potential against gram-positive pathogens[4]. However, the additional outer membrane (OM) of gram-negative bacteria provides an impermeable barrier and prevents endolysins from accessing the PG. To overcome the OM, we propose the fusion of other phage proteins to endolysins to generate phage-inspired antimicrobial enzymes that we term 'Phagezymes'. Libraries of variant proteins were created using the DNA shuffling method, VersaTile[5], to 'mix-and-match' phage 'parts' for screening of antimicrobial potential against *Psa*. We will present our work into the use of phage proteins as alternative antimicrobials.

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ID49: Mapping interactions between Group A Streptococcus (GAS) M-protein and antibodies

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Group A Streptococcus (GAS) is an obligate human pathogen associated with a broad spectrum of disease from mild pharyngitis (Strep throat) to serious invasive disease and post-infectious diseases such as post streptococcal glomerulonephritis and acute rheumatic fever (ARF). ARF remains a major disease burden in New Zealand, almost exclusively affecting Māori and Pacific children. The M-protein is a major GAS virulence factor expressed on the surface of bacterium. It has a central role in colonisation, is an immune evasion factor, and a leading target for vaccine development. It is also a diverse protein and the basis for GAS strain typing with over 200 *emm*-types identified to date. Despite the importance of the protein, there is a lack of molecular understanding of antibody mediated protection induced by the M-protein. This is complicated by studies that have shown that M-protein can bind antibodies non-specifically via the Fc region as part of its immune evasion function. The current study aims to provide structural understanding about how a representative M protein (M12) interacts with immune recognition molecules. To achieve this aim, nanobodies specific to M12 protein were generated following vaccination of Llamas with full-length M12 protein. The resulting M12 specific nanobody library was screened and two nanobodies that interact with M12 protein were selected for further characterisation. These analyses, including specificity, affinity, and the ability to induce opsonophagocytic killing of GAS will be presented.

ID50: Characterising the Immune Response in Acute Rheumatic Fever following Hydroxychloroquine Treatment

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Acute Rheumatic Fever (ARF) is an autoimmune sequela to Group A Streptococcus (GAS) infection that disproportionately affects Māori and Pacific children, who are 20-40 times more likely to develop the disease than other children in NZ(1). There are no effective immunomodulatory treatments available for ARF, increasing the urgency of investigation into potential therapies. Hydroxycholoroquine (HCQ) is an anti-inflammatory drug used to treat multiple autoimmune diseases, and was demonstrated *in vitro* to suppress the dysregulation of a pro-inflammatory IL-1β-GM-CSF cytokine axis associated with ARF(2). It was recently shown to be safe and associated with control of inflammatory markers *in vivo* in two ARF patients in New Zealand(3). An observational trial is now underway in Auckland (ethics #20/NTB/163) to assess the safety and suppression of inflammation by HCQ in ARF. An immunological sub-study is being performed to characterise the 'immunophenotype' of ARF patients before and after HCQ treatment. This includes spectral flow cytometry analysis of peripheral blood mononuclear cells (PBMC) and multiplex serum cytokine profiling. This will allow temporal investigation of HCQ mediated immune modulation, and identification of ARF immune features. To confirm this *ex vivo* analysis, *in vitro* PBMC stimulation assays will be performed to characterise responses to GAS exposure in these ARF patients over the duration of HCQ treatment. This combined immunological analysis will support the validation of HCQ as a possible therapeutic agent for ARF patients and inform disease pathogenesis.

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ID51: Disrupting Bacterial Metal Ion Homeostasis to Break Antimicrobial Resistance

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Methicillin-resistant Staphylococcus aureus (MRSA) is a multi-drug resistant (MDR) opportunistic human pathogen, which produces an alternative penicillin-binding protein (PBP2a), rendering it resistant to β-lactam antibiotics. Therefore, the World Health Organisation has classified MRSA as a 'high priority' pathogen where novel antibacterial treatments are urgently required. The reversal of antimicrobial resistance by antibiotic adjuvants is a promising strategy to restore the activity of antibiotics against MDR bacteria. We have demonstrated that the zinc ionophore PBT2, alongside Zn (PBT2-Zn), can act as an antibiotic adjuvant, resensitising multiple MDR bacteria to killing by otherwise ineffective antibiotics. However, the molecular mechanisms underlying this resensitisation are currently unknown. To study this phenomenon, we investigated the ability of subtherapeutic PBT2-Zn to restore the bactericidal activity of the β-lactam oxacillin in vitro in the communityassociated MRSA isolate USA300. We observed that PBT2-Zn combined with oxacillin dysregulated metal ion homeostasis in USA300 through the intracellular accumulation of zinc ions and reduced intracellular manganese ions. We also observed that manganese supplementation rescued USA300 from the bactericidal killing by PBT2-Zn-oxacillin treatment. The expression of several critical genes involved in β-lactam resistance were upregulated in response to PBT2-Zn-oxacillin, including mecA, which encodes PBP2a. In contrast, critical genes involved in virulence (e.g., saeS) and manganese transport (e.g., mntA) were downregulated. This study demonstrates that disruptions in bacterial metal ion homeostasis can trigger several genetic and biochemical changes in MRSA USA300, resulting in antibiotic sensitisation. Our investigations form the basis for further research to reveal the molecular and metabolic mechanisms that underpin antibiotic resistance reversal, and how PBT2 is able to function as an adjuvant against diverse bacterial species. These data could enable us to design novel antibiotic adjuvants to sensitise a broad range of resistant bacteria to different antibiotic classes.

ID52: Investigation of antimicrobial tolerance in *Enterococcus faecalis*

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Multidrug resistance in enterococci is a rising clinical concern with failure to treat infections leading to higher mortality rates. *Enterococcus faecalis* is a leading cause of hospital-acquired infections with known antimicrobial resistance (AMR) and tolerance. Resistance and tolerance are important mechanisms in allowing bacteria to avoid the bactericidal effect of antimicrobials. Tolerance often involves reduced or dormant (non-replicative) growth rates which aids in survival at high antimicrobial concentrations. Increased survival in the presence of antibiotic provides an opportunity for mutations to occur that can lead to AMR. The molecular mechanisms and impact of tolerance are still relatively unknown, therefore the aim of this project was to identify genes and pathways of antimicrobial tolerance using *Enterococcus faecalis* as a model system. This involved the screening of a previously established *E. faecalis* transposon mutant library (8000 mutants, 4-fold coverage) for ampicillin and daptomycin loss and gain of tolerance mutants (1). Antimicrobial susceptibility assays were used to optimise concentrations required to identify loss and gain of tolerance mutants in the *E. faecalis* transposon mutant library. A high-throughput microtiter 96-well plate method using resazurin as an indicator of cell viability was used to screen the library. Antimicrobial concentrations of 4 µg/mL and 64 µg/mL, and 2 µg/mL and 16 µg/mL were identified as optimal concentrations for screening of loss and gain of tolerance mutants for daptomycin and ampicillin, respectively. The use of transposon mutagenesis allows for the identification of genes related to antimicrobial tolerance. It was hypothesised that exposure to chosen antibiotics, mutants conferring tolerance would be identified. Approximately 10 potential tolerant mutants have been isolated thus far which require further investigation. Whole genome sequencing and confirmation through antimicrobial susceptibility assays will be undertaken with the isolated mutants to identify significant genes and pathways. Overall this will improve the understanding of antimicrobial tolerance and key genetic components in the bacterial pathogen *E. faecalis*.

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ID53: The anti-viral role of host class II histone deacetylases in influenza A virus infection

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Influenza A virus (IAV) is a primordial acute respiratory illness affecting 10% of the global population annually[1]. IAV interacts with a plethora of host factors in each stage of its replication[2]. Host acetylation plays a crucial role during IAV infection and different classes of histone deacetylases (HDACs) are central to negatively regulating the acetylation level. Recent findings exemplified that HDAC4 and HDAC6 of the class II have a strong antiviral role in IAV replication[3,4]. Since it is imperative to individually define the role of each HDAC in IAV infection, we hypothesized that the rest of Class II [a (5,7,9) and b (10)] have a role in IAV antagonism and vice versa. We demonstrated that the rest of class II have a potential antiviral role in the virus replication by RNAi screening and ectopic expression. Indeed, we observed that IAV replication was elevated in depleted cells and plummeting in supplemented cells. In turn, IAV strongly antagonize these HDACs at the mRNA and polypeptide level (HDAC10) via IAV vRdRp (PA-X, PA, PB1 and PB2) in a time, dose, strain independent and cell line dependent manner. We also established a physiologically relevant model of human airway (Air-liquid interface model) and observed a similar anti-viral potency. These results demonstrate the potent anti-viral role of HDACs and could be promising factors in the anti-influenza arsenal. Further, analysis of IAV-induced global host transcriptome and proteome analysis could delineate the anti-IAV mechanism of these HDACs.

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ID54: Multiplex transcriptional repression to investigate genetic interactions between bioenergetic complexes in *Mycobacterium tuberculosis*

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New drug targets and drug combinations for *Mycobacterium tuberculosis* are urgently required. Drug combinations that target the mycobacterial respiration have shown promise in combatting antimicrobial resistance. Advancing this target space further has been slowed by a lack of on-target inhibitors for the majority of respiratory complexes and an incomplete understanding of which inhibitor combinations have the strongest interactions. To overcome these limitations, we have used CRISPR interference (CRISPRi) to characterize the consequences of transcriptionally inhibiting individual respiratory complexes in *Mycobacterium tuberculosis* and identify bioenergetic complexes that when simultaneously inhibited, result in rapid cell death. Single, double, triple, and quadruple gene knockdowns of multiple respiratory chain components were constructed and used to probe gene essentiality and consequences of inhibition.

We identified a number of new gene-gene interactions that when transcriptionally silenced caused growth inhibition (essentiality) and rapid cell death (cidality). For example, the simultaneous transcriptional repression of *qcrB* and *cydB* resulted in a bactericidal phenotype, indicative of a synthetically lethal interaction. In summary, these results provide fundamental insights into the functions of and interactions between bioenergetic complexes and the utility of CRISPRi in designing new drug combinations.

ID55: The Effect of Needle Length and Arm Size in mRNA COVID-19 Immunization

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The mRNA COVID-19 vaccine, Comirnaty™/BNT162b2, is licensed for intramuscular injection. We investigated the relationship between needle length and the skin to deltoid muscle distance (SDMD) and its effect on spike antibody levels following one dose of BNT162b2. Adults who had received their first dose of BNT162b2 had their SDMD measured by ultrasound, and anthropometric and needle length measurements collected. Finger-prick blood samples were collected at baseline (first Dose) and when participants returned for their second dose (3-6 weeks later) to measure spike antibody levels. Three groups were identified based on needle length and SDMD: 'clearly sufficient' (needle exceeding SDMD by >5 mm), 'probably sufficient' (needle exceeding SDMD by ≤ 5 mm), and 'insufficient' (needle length ≤ SDMD).

There were 402 participants recruited with a mean BMI of 29.1 kg/m^2, arm circumference of 37.5 cm and SDMD of 13.3 mm. A small proportion (23/402, 5.7%) had a SDMD >25 mm, the length of a standard injection needle. Both arm circumference (≥40 cm) and BMI (≥33 kg/m^2) could identify those with a SDMD of >25 mm, with a sensitivity of 100% and specificities of 71.2 and 79.9%, respectively. Paired blood samples were collected for 249/402 (62%) participants and showed no significant difference in spike antibody titres between the three needle length groups. The mean spike Binding Antibody Units/mL (BAU/mL) was not significant between the three groups: 464.5 BAU/mL in 'clearly sufficient' (n = 217), 506.4 BAU/mL in 'probably sufficient' (n = 21), and 489.4 in 'insufficient needle (n = 11).

A standard 25 mm needle is likely to be insufficient to deliver the vaccine into the deltoid muscle in a small proportion of adults with larger arms. However, spike antibody titres were not significantly lower in this group, suggesting vaccine deposition into the deltoid muscle may not be necessary for mRNA vaccines.

ID56: Elevated IgG3 in Acute Rheumatic Fever

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Acute Rheumatic Fever (ARF) is a serious autoimmune-mediated inflammatory disease that can develop after infection with group A *streptococcus* (GAS; *Streptococcus pyogenes*) and can lead to permanent heart valve damage and rheumatic heart disease (RHD). ARF incidence continues to be unacceptably high in Māori and Pacific youth[1,2]. In a previous systems immunology analysis, we observed that immunoglobulins of the IgG3 subclass are significantly elevated in ARF patient serum, with >90% having levels above clinical reference ranges[3]. The aim of this project is to investigate possible causes and consequences of this striking elevation. IgG3 is the most potent immunoglobulin subclass with a wide range of effector functions. It plays an important role in clearing bacterial infections, however when dysregulated, it contributes to symptoms in other autoimmune diseases. In general, IgG3 has a reduced half-life (7 days) compared to IgG1 (21 days), but a specific IgG3 allotype containing a p.Arg435His variation has a prolonged half-life[4]. The presence of IgG3(Arg435) or IgG3(His435) was quantified in sera obtained from recently completed large-scale studies of ARF and GAS infections in New Zealand[3,5] (62 ARF cases, 30 GAS pharyngitis, and 34 controls) using indirect enzyme-linked immunosorbent assays (ELISAs). No samples indicated a positive signal for IgG3(His435), suggesting this allotype is not contributing to elevated IgG3 levels. Next, antigen specific responses were explored using immunoassays with antigens present on the GAS surface (M-proteins), or secreted antigens (streptolysin-O). Elevations in IgG3 responses to M-proteins were observed in all groups compared to streptolysin-O. However, this was significantly more exaggerated in ARF compared with GAS pharyngitis ($p<0.01$). This points to a role for excessive IgG3 responses following GAS infection, which may contribute to a harmful inflammatory response in ARF. Future work will investigate Fc-receptor engagement of serological IgG3 to assess the role of immunoglobulin effector functions in the disease.

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ID57: The molecular clock contributes to the host response to infection through regulating the complement system.

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Circadian rhythms help to synchronise physiological processes with daily environmental changes. The immune response to bacterial infection exhibits circadian rhythmicity, characterised by elevated anti-bacterial activity during an organism's active phase, when the threat of infection is greatest. At the molecular level, oscillations in immune gene expression are believed to be controlled by molecular clocks that operate in cells of the immune system. The complement system provides a crucial frontline defence mechanism against bacteria. Whether expression of complement components in the liver is regulated by the molecular clock remains unknown.

We have generated a zebrafish knockout line for the light-responsive clock gene *per2* that demonstrates decreased survival following infection and reduced expression of *c3a.3* (a zebrafish orthologue to the complement component *C3*). Supporting a bactericidal function for C3a.3, *c3a.3*$^{-/-}$ larvae are deficient in clearing injected bacteria and are more susceptible to infection. We show that steady-state expression levels of *per2* within the liver, but not *c3a.3*, demonstrate time-of-day variation, with elevated levels during the active (light) phase. This is in contrast to the infected state, where infection-induced expression of *c3a.3* does demonstrate time-of-day variation, with highest levels observed when infections are delivered during the active (light) phase. This work reveals that the core clock component Per2 contributes to the host response to infection through positively regulating *c3a.3* expression in the liver and provides novel mechanistic insights connecting the molecular clock and the complement system.

ID58: The interplay between GAS virulence factors and a functional antibody response.

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Streptococcus A (StrepA) is an obligate human pathogen that causes a range of diseases globally, ranging from superficial pharyngitis and skin infections, to invasive disease and secondary auto-immune diseases like acute rheumatic fever. There are no licensed vaccines for StrepA currently available. Two leading vaccine candidates are based on the M-protein and T-antigen cell surface proteins. The M-protein is a hypervariable surface protein and functions as a major immune evasion molecule. It also forms the basis for strain typing, with over 200-*emm* types identified to date. In contrast, the T-antigen is more conserved between strains with just 18 major *tee*-types identified globally. The T-antigen functions as the backbone subunit of the StrepA pili and is polymerised enabling vast extension beyond the bacterial cell surface and capsule. While the M-protein and T-antigen are being developed as distinct vaccine candidates, there is a gap in understanding as to whether expression of either antigen impacts a protective immune response to the other during infection. Accordingly, the aim of this project is to investigate how M-protein and T-antigen expression levels influence opsonophagocytosis of StrepA in the presence of M- and T-specific antisera. The sera has been characterised using immunoassays, and antigen expression on StrepA quantified by flow cytometry. Data from opsonophagocytic killing assays performed with M- and T-specific antisera alone and in combination, correlated with antigen affinity and expression levels, will be presented.

ID59: Investigating the functional role respiratory oxidases play in the bacterial pathogen *Enterococcus faecalis*

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Enterococcus faecalis is an opportunistic pathogen and a significant source of hospital-acquired infections (1). Multidrug-resistant enterococci are notoriously difficult to treat due to their intrinsic resistance and tolerance to a wide range of antibiotics. A key molecular determinant of *E. faecalis* antimicrobial tolerance is the two-component system CroRS (2). It is hypothesized that CroRS mediates tolerance by controlling the cellular response to antimicrobial-induced production of reactive oxygen species (ROS) (3, 4). *E. faecalis* is an facultative anaerobe, and appears to have two major enzymes to combat oxidative stress under aerobic conditions, the NADH oxidase (Nox), and cytochrome bd oxidase (CydAB). These two enzymes are part of the CroRS-induced regulon. The functional role of respiratory oxidases is an unexplored field in enterococcal research and understanding the fundamental link to antimicrobial tolerance in *E. faecalis* will advance our molecular understanding of these mechanisms. We hypothesize that regulation of oxidase enzymes in response to antimicrobial stress has a key role in antimicrobial tolerance in enterococci. Single gene knockout strains were made to identify the functional role of each oxidase enzyme in *E. faecalis*. Oxygen consumption rates were determined in the oxidase knockout strains and compared to the isogenic wildtype. The transcriptional profile of these oxidase genes in the presence and absence of antimicrobial stress was elucidated in the wildtype and a *ΔcroRS* knockout using quantitative real-time PCR. Transcriptional expression of both oxidase genes identified a dynamic inverse relationship, which was enhanced under vancomycin-induced stress. In response to vancomycin stress CroRS upregulates *nox* but downregulates *cydAB*. Nox was identified as the dominant mechanism of oxygen consumption. Oxygen consumption in *E. faecalis* is primarily facilitated by Nox, however this alone does not impact antimicrobial tolerance. Each oxidase does not impact tolerance independently, indicating that complex regulation between oxidases is the important determent of antimicrobial tolerance.

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ID60: Modelling Covid-19 like disease Spread Using Real Transport Flow

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The world was struck by the SARS-CoV-2 pandemic, at the beginning of 2020. Computational epidemiological modeling became an important asset to predict the emergence and propagation of the pathogen. Travel restrictions are the best measure for ceasing the propagation of pathogens. For an effective implementation of such interventions we need a better understanding of the effects and benefits of such restrictions a priori. This calls for a metapopulation modeling that incorporates population exchange between different locations. Basic metapopulation models do not identify individuals according to their home locations and formulate the problem as Markovian processes. However, the primary pattern of transportation is commuting from home to work and the reverse commuting. Thus, it is important to label the commuters according to their original locations. Hence we developed a metapopulation susceptible exposed-infected recovered (SEIR) compartment model for commuting individuals in a population, using population and mobility data at the city level, without keeping track of each individual separately. We used the population flow data from China to constrain the parameters in our model. And we conducted an investigation on whether there is a relationship between commuting frequency (the return rate) and infectious period, Basic reproduction number R0, & incubation period of the infection? We identified some pivot locations in China with the help of PageRank algorithm and real population flow data. Finally, we compared the SEIR disease dynamics in the entire country by simulating interventions on pivot cities.

ID61: Feasibility of using a luminescence-based method to determine serum bactericidal activity against *Neisseria gonorrhoeae*

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Development of a vaccine to limit the impact of antibiotic resistant *Neisseria gonorrhoeae* is a global priority. Serum bactericidal antibody (SBA) is a potential indicator of protective immunity to *N. gonorrhoeae*, but conventional assays measuring colony forming units (CFU) are time-consuming and labour intensive. We determined the feasibility of measuring viability of *N. gonorrhoeae* using a high throughput, same day luminescent assay.

The BacTiter-Glo™ Microbial Cell Viability Assay (ATP assay) was modified for use in estimating viable bacterial numbers. The luminescent signal rose with increased *N. gonorrhoeae* CFU across a range of 50 – $5x10^6$ CFU, with a strong correlation between CFU and luminescence for all strains (r=0.9). Normal Human Serum (NHS) was screened for serum sensitivity and murine antibodies to outer membrane preparations used to determine bactericidal titres in parallel to CFU readings. NHS from 10 individuals were used for serum sensitivity assays - sensitivity values were significantly reduced with the ATP method for *N. gonorrhoeae* strains FA1090 (5/10, $p < 0.05$) and MS11 (10/10, $p < 0.05$), whereas P9-17 data were comparable for all donors. However, mouse anti-P9-17 outer membrane vesicles (OMV) SBA titres to P9-17 were comparable with both methods ($r = 0.97$).

Detection and quantification of *N. gonorrhoeae* ATP using a commercially available luminescent substrate shows utility as an alternative approach to manual enumeration of CFU for measuring SBA to *N. gonorrhoeae*. In contrast, screening of NHS from multiple donors using the ATP method under-estimated serum sensitivity of the FA1090 and MS11 strains relative to the CFU method, suggesting that it is unsuited to this assay. We are currently applying this method to additional strains of *N. gonorrhoeae*. The ATP assay may be advantageous for directly reading antibody-mediated killing, independent of alternative mechanisms that kill the gonococci over a longer period of time.

ID62: Metabolic Dysregulation in Drug-resistant *Mycobacterium tuberculosis*

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Tuberculosis (TB) is among the top infectious diseases worldwide, causing an estimated 1.4 million death in 2019. Although multiple drugs, such as rifampicin and isoniazid, have been used at treating the causative agent of tuberculosis (i.e. *Mycobacterium tuberculosis*), several antimicrobial-resistant strains have been identified. Because treatment for drug-resistant strains of *M. tuberculosis* is limited, leading to longer treatment time (< 6 months for drug-susceptible strains vs >18 months for drug-resistant strains) and significant cost, causing a huge burden on the medical resources. This highlights the urgency for developing novel treatments to rapidly eliminate and reduce the emergence of drug-resistant *M. tuberculosis* strains.

In this project, genome-wide screening results via the CRISPR interference (CRISPRi) transcriptional knockdown system were compared between the drug-susceptible $mc^2$6206 and its drug-resistant derivatives (i.e. INH-resistant, RIF-resistant, and BDQ-resistant). Fold-change analyses were conducted to identify the metabolic impacts of drug resistance in *M. tuberculosis*. With a 2-fold change cut-off and adjusted p-value ≤ 0.01, We identified over 700 vulnerable genes in various processes, such as cell wall synthesis, respiration, and lipid metabolism. Our results identified vulnerable genes in drug-resistant *M. tuberculosis* strains, providing high-value targets for novel treatment development.

ID63: Can BCG vaccination train innate immune cells to protect against skin and soft tissue infections?

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Skin and soft tissue infections (SSTIs) continue to be a major health concern in NZ with SSTI hospitalisation rates in children almost 8 times higher than in other developed countries[1]. Māori and Pacific children are most affected. Increasing antibiotic resistance provides additional challenges, with fewer antibiotics available to treat the two main bacterial causes of SSTIs, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Bacillus Calmette-Guérin (BCG) vaccination is used worldwide to reduce the transmission of the causative agent of tuberculosis, *Mycobacterium tuberculosis.* It has been observed that the BCG vaccine has heterologous effects against other antigenically diverse infections, reducing all-cause mortality in neonates. The mechanism underpinning this is "training" of innate immune cells to enhance antimicrobial activities to confer broad protection against a range of diverse pathogens[2]. We hypothesised that the BCG vaccine might reduce the severity of SSTIs in a mouse wound model through the induction of trained innate immunity. C57BL/6 mice were vaccinated subcutaneously or intravenously with BCG, then four to eight weeks later given a high-density *Pseudomonas aeruginosa* or *Staphylococcus aureus* skin infection on the right dorsum. Abscess wound area and depth was calculated daily. Mice were culled 2 or 7 days post-infection, spleen and wound-draining lymph nodes excised (day 2), and the wound excised and plated to quantify bacteria (day 7). Lymphocyte and phagocyte responses in the draining lymph nodes and spleen were evaluated by flow cytometry. Contrary to our hypothesis, we found that BCG vaccination increased the severity of both *P. aeruginosa* and *S. aureus* skin infections. Both wound size and bacterial numbers were increased in mice that had received the BCG vaccine, compared to unvaccinated mice. This raises the possibility that the BCG vaccine may increase the risk for severe SSTI. The immunological mechanisms driving this deleterious response are being investigated.

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ID64: The threat of carbapenem resistant *Acinetobacter baumannii* in Fiji.

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Carbapenem resistant *Acinetobacter baumannii* (CR-Ab) is as a pathogen of critical concern. This is because the pathogen has the ability to resist multiple classes of antimicrobials, including last resort antimicrobials such as carbapenems, and mobilise antimicrobial resistance genes within or between strains and species, and contribute enormously to increased healthcare costs, morbidity, and mortality

Little is known about the nature of transmission and resistance of *A. baumannii* in Fiji. Using whole genome sequencing (WGS), we confirmed two prolonged parallel undetected outbreaks of nosocomial infection associated with the international high risk clone CR-Ab sequence type 2 (ST2) at the CWMH in 2019. The two outbreaks were derived from two groups of genetically closely related CR-Ab ST2 isolates. All isolates within the two outbreaks harboured a wide range of antimicrobial resistance genes, with carbapenem resistance being predominantly mediated by $bla_{OXA\text{-}23}$ and $bla_{OXA\text{-}66}$ genes. We found that group 1 of the 2019 CWMH outbreak clone were genetically related to the historic 2016 – 2017 outbreak at CMWH suggesting that this outbreak clone has been there for more than three years. Moreover, these outbreak clone shared a common ancestral linkage to the CR-Ab ST2 isolated from a Brisbane hospital outbreak in 2016 to 2018 suggesting a potential Oceania CR-Ab ST2 strain. We also showed that he second outbreak group also shared common ancestry to India for which Fiji shared strong medical linkages with. While we cannot definitively identify the source, the direct linkage of groups 1 and 2 to Australian and India strains respectively, suggests that international travel may have contributed substantially to the spread and acquisition of CR-Ab ST2.

These findings underscore the need for an ongoing "enhanced" surveillance and improvement in antimicrobial stewardship and extra infection prevention and control precautions. Moreover, we demonstrated the power of WGS in response to outbreak detection and the detail in which the threats are understood, ultimately leading to quicker and more targeted interventions.

ID65: Exploiting metabolic dysregulation to potentiate antibiotic killing of resistant *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is an obligate human pathogen and causative agent of tuberculosis (TB). TB is a leading cause of mortality by infectious disease and, although curable, many antibiotics are rendered useless due to antibiotic resistance. Because of this, it is of major importance that therapeutic strategies to potentiate antibiotic lethality and rapidly sterilize antibiotic-resistant *M. tuberculosis* are developed.

Antibiotics function by producing primary and secondary stressors. Primary stress involves interactions with molecular targets, which result in the inhibition of bacterial growth. Secondary stressors involve the downstream consequences of target inhibition, including metabolic dysregulation and the production of reactive oxidants that are responsible for influencing antibiotic lethality.

Genetic mutations that confer resistance to an antibiotic frequently occur in processes essential for cellular function, and result in poorly characterized metabolic consequences that are commonly observed as a fitness loss or growth impairment. As antibiotic efficacy is intrinsically linked to metabolism, we hypothesize that antibiotics that exploit metabolic dysregulation will rapidly kill antibiotic-resistant strains of *M. tuberculosis*.

Here we will present progress towards understanding the metabolic consequences of drug-resistance in *M. tuberculosis* and how these changes can be used to rapidly kill drug resistant strains. Metabolic consequences are being detected using metabolomics which captures the metabolic state of a cell population. Findings from this research will increase the repertoire of TB treatments and improve the outcome of treatment of antibiotic-resistant *M. tuberculosis*.

ID66: Development of a COVID-19 rapid antigen test using filamentous phage-derived nanorods as detector particles

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The COVID-19 pandemic has caused illness and death worldwide, significantly disrupting everyday life. To cope with the pandemic caused by a highly transmissible virus, an easy-to-use antigen detection test with a quick turnover time is vital to allow timely diagnosis of infectious individuals and subsequent isolation, terminating the chain of transmission. Here we employ Ff-filamentous phage-derived nanorods displaying an scFv specific to SARS-CoV-2 spike protein to detect the virus in different formats of immunoassays. The nanorods were enzymatically conjugated, using a purified transpeptidase Sortase A, with biotin, to allow colorimetric detection with streptavidin-alkaline phosphatase. Biotinylated nanorods were able to detect purified SARS CoV-2 spike protein in dot blots and ELISA assays, when used in combination with the monoclonal antibody CR3022 as its partner capture agent. Notably, the sensitivity of the ELISA format can reach as low as 1 pg/µL, equivalent to approximately 10^4 virions/µL. We are currently adapting our nanoparticles to detect another SARS-CoV-2 antigen due to the high occurrence of mutations within the spike protein. These nanoparticles will then be used to develop lateral flow assay that can be easily carried out by users at home, with a low cost and the result available in minutes. This type of testing has proved to be an essential public health tool for widespread use, when testing demand exceeds laboratory capacity, along with frequent screening of individuals to prevent outbreaks within workplaces, health care and education facilities.

ID67: Using antibiotic combinations to suppress development of resistance

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Pseudomonas aeruginosa causes long-lasting, intractable infections in patients with chronic lung diseases, such as cystic fibrosis, and is a leading cause of hospital-acquired infections. The cephalosporin β-lactam antibiotic ceftazidime is commonly used to treat *P. aeruginosa* infections but bacteria can become resistant through mutations or acquisition of β-lactamases (enzymes that degrade β-lactam antibiotics). Strategies to prevent the emergence of resistance are therefore urgently needed. Here we show that ceftazidime can act synergistically with the macrolide antibiotic azithromycin to increase the effectiveness of both antibiotics and suppress the development of resistance *in vitro*. Ceftazidime also acts synergistically with the aminoglycoside tobramycin and the polymyxin colistin. These combinations may be useful clinically to improve treatment efficacy and reduce resistance development. However, testing in standard culture media does not represent the conditions under which bacteria grow in a patient, nor account for the host immune effects, potentially masking changes that might alter antibiotic effectiveness during infection. We show that minimum inhibitory concentrations of antibiotics can differ by up to ten-fold between bacterial culture media and tissue culture media. Further, we are testing whether the synergism of these antibiotics is retained in tissue culture media, and, to most accurately recapitulate the conditions bacteria are exposed to in an infection, also in a chronic murine subcutaneous *P. aeruginosa* infection model. Collectively our findings will provide a thorough understanding of how antibiotic combinations act together in clinically relevant conditions. Such knowledge can inform rational combinatorial treatment of *P. aeruginosa* infections in patients, improving outcomes and suppressing the emergence of antibiotic resistance.

ID68: Post infection cellular and humoral immune responses of New Zealanders to SARS-COV-2

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the current pandemic and is responsible for over 3.89 million deaths worldwide. During a viral infection both the innate and adaptive immune systems play critical roles in defending the host against the virus and clearing it. Defining SARS-CoV-2-specific adaptive immune responses following infection is important for understanding protection against reinfection and for vaccine design. In this study, we defined the humoral and T cell responses in a cohort of patients following infection with SARS-CoV-2 in the absence of re-exposure to the virus.

Serum and peripheral blood mononuclear cells were collected from convalescent patients who had previously tested positive by RT-PCR for SARS-CoV-2. To measure neutralising antibodies, pseudotyped virus neutralization assays were performed at various serum dilutions and the IC50 was determined. SARS-CoV-2 specific CD4+ and CD8+ T cell responses were assessed by activation induced marker (AIM) and proliferation assays.

Analyses are ongoing, and the results will be presented at the meeting.

ID69: Serine acetyltransferase from *Neisseria gonorrhoeae*; structural and biochemical basis of inhibition

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Neisseria gonorrhoeae is an obligate human pathogen and the causative agent of the sexually transmitted infection, gonorrhoea. Over decades of antibiotic use, gonorrhoea has evolved antimicrobial resistance at an alarming rate and has reduced treatment to a sole antibiotic therapy, which recently has also failed to treat gonorrhoea infection. Overcoming this rapid emergence of antibiotic-resistant *N. gonorrhoeae* requires the development of new antimicrobials to secure successful treatment of gonorrhoea in the future.

Biosynthesis of the amino acid, cysteine, has been identified as a promising pathway for developing these new antimicrobials. Cysteine is not only essential for protein folding, but is also an essential precursor for redox compounds, such as glutathione, which is key for N. gonorrhoeae's ability to mitigate oxidative stress upon infection. Serine acetyltransferase (CysE) catalyses the first step in the two-step cysteine biosynthesis pathway and is an essential gene in *N. gonorrhoeae*. Using X-ray crystallography, we have determined the structure of the CysE with and without substrate bound. Structural insights show that CysE shares conserved structural features and active site residues, with the acyltransferase protein family. These results, along with recently collected CysE kinetic data demonstrate that CysE from *N. gonorrhoeae* is a functional serine acetyltransferase and is sensitive to feedback inhibition by the pathway product, cysteine. These findings put us in a unique position to develop inhibitors to this key enzyme to overcome extensively antimicrobial resistant *N. gonorrhoeae*.

Oldham, K. E. A., Prentice, E. J., Summers, E. L., & Hicks, J. L. (2022). *Serine acetyltransferase from Neisseria gonorrhoeae; structural and biochemical basis of inhibition.* Biochemical Journal, 479(1), 57-74. doi:10.1042/bcj20210564

ID70: Investigation of a causative agent for Respiratory Distress Syndrome in neonatal hoiho

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Hoiho/yellow-eyed penguins (*Megadyptes antipodes*) are predicted to become extinct on mainland Aotearoa New Zealand in the next few decades, with disease being a significant contributor to their decline[1,2]. More recently, a new disease phenomenon termed respiratory distress syndrome causing lung pathology has been identified in very young chicks, however no causative pathogens have been identified. In 2020 and 2021, the number of deaths of chicks from this condition increased four- and fivefold, respectively, with a mortality of >90% and most chicks succumbing to death within the first week of life. This project aimed to identify possible pathogens responsible for disease impacting hoiho. Total RNA was extracted from tissue samples (liver, lung, kidney, spleen +/- bursa of fabricus) collected during post-mortem of 43 chicks and subject to meta-transcriptomic sequencing to examine for the presence of bacteria, fungi, protozoa, DNA viruses and RNA viruses. To this end, we identified a novel, highly divergent and abundant Gyrovirus, which shared only 40% amino acid identity within VP1 with another Gyrovirus discovered in a diseased seabird[3]. Due to the high relative abundance of viral reads, it is highly likely that this novel Gyrovirus is associated with disease in these chicks. Further prevalence estimates in other seabird populations are needed to assess potential reservoirs of this virus. Finally, preventative therapy such as the development of vaccines could be useful in protecting these endangered wildlife.

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2 Mattern T, M. S., Ellenberg U, Houston DM, Darby JT, Young M, van Heezik Y, Seddon PJ. *Quantifying climate change impacts emphasises the importance of managing regional threats in the endangered Yellow-eyed penguin*. PeerJ **5**, e3272, doi:https://doi.org/10.7717/peerj.3272 (2017).

3 Li, L. et al*. A gyrovirus infecting a sea bird.* Archives of Virology **160**, 2105-2109, doi:10.1007/s00705-015-2468-1 (2015).

ID71: Structural and biochemical characterisation of *O*-acetylserine sulphydrylase (CysK) from *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is the causative bacteria of the sexually transmitted infection, gonorrhoea. Rapid emergence of antibiotic resistant strains of *N. gonorrhoeae* has given rise to urgent need for development of new antimicrobial treatments. Cysteine plays a vital role in protein folding and function, and synthesis of glutathione for protection against oxidative stress during infection, making it a promising new target for development of antimicrobials. *O*-acetylserine sulphydrylase (OASS) catalyses the final step of the two-step cysteine biosynthesis pathway. Most bacteria have two isoforms of OASS that produce cysteine, sulphide and *O*-acetylserine utilising OASS-A/CysK, and thiosulphate utilising OASS-B/CysM. *N. gonorrhoeae* has only the OASS-A/CysK isoform in its genome. This research characterises the structure and function of CysK to determine its role in *N. gonorrhoeae's* unique cysteine biosynthesis pathway. Kinetic characterisation demonstrates CysK has OASS activity, displaying positive cooperativity with respect to substrates, O-acetylserine and sulphide. Sulphide shows partial allosteric inhibition, and thiosulphate is not used as a substrate. The CysK structure was solved to 2.49 Å by X-ray crystallography and shows CysK belongs to the tryptophan synthase β-superfamily and adopts a homo-dimeric structure consisting of two monomers. Positive cooperativity is supported as co-factor binding residues are in inactive and active conformations in each monomer of the dimer, respectively. It is still uncertain whether CysE and CysK from *N. gonorrhoeae* are able to form a complex, the presence or lack of which has interesting implications for regulation of sulphur flux and cysteine production. This research is a major leap in our understanding of the uncharacterised cysteine biosynthesis pathway in *N. gonorrhoeae*.

ID72: Towards a New Class of Anti-Tuberculosis Drugs: Design and Synthesis of AnPRT Transition State Analogues

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Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb)*. Globally, it is the second biggest cause of death from a single infectious agent, after COVID-19, with an estimated 1.5 million deaths in 2020. Despite the fact that drug-susceptible TB can be cured using a combination of antibiotics over several months, the treatment has barely changed over the last 40 years. This has given rise to antibiotic-resistant strains with an estimated 150,000 cases that were resistant to at least one of the first line drugs in 2020 alone. This highlights a clear need for a new class of anti-TB drugs that will be able to tackle this growing health crisis.[1]
Our project aims to design new drugs that will target an underexploited biosynthetic pathway in *Mtb.* The Tryptophan (Trp) biosynthetic pathway is responsible for the production of Trp, an amino acid essential for the survival and virulence of *Mtb*.[2] This pathway is absent in mammals making it an ideal candidate for drug development. Our aim is to design and synthesise enzyme inhibitors of anthranilate phosphoribosyl transferase (AnPRT), an enzyme involved in the Trp pathway.

Transition-state analysis (TSA) is a sophisticated technique to design potent enzyme inhibitors. This method is exemplified by Mundesine®, a drug used for the treatment of peripheral T-cell lymphoma in Japan. This presentation will cover this project that builds on the Ferrier Research Institute's experience and success in TSA and knowledge of the AnPRT enzyme to design inhibitors that could be critical in the fight against TB.[3]

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ID73: Understanding and Targeting Tryptophan Biosynthesis in Human Pathogens

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Tuberculosis (TB) remains a prevalent disease throughout the worlds effecting tens of millions of people annually. The current compliment of pharmaceuticals used to treat TB infections continues to lose ground against the ever-increasing prevalence of single and multi-drug resistant strains. This has prompted the development of new, highly potent, selective drugs to target TB. To this end, the anthranilate phosphoribosyltransferase (AnPRT) enzyme is being examined as a target for new anti-TB agents. AnPRT catalyses a commitment step early in the biosynthesis of tryptophan which is critical for TB growth and survival. The mechanism of AnPRT is probed using kinetic isotope effects in concert with high level calculations to determine the structure of the reaction transition state. Transition state inhibitors are being and will be designed to target this transition state and in turn prevent tryptophan biosynthesis killing the TB microbacterium without harming the human host. This presentation will cover the expression, purification, and preliminary kinetic studies around AnPRT including the design of kinetic experiments with both cold and hot substrates. Further, the in-house synthesis of radiolabelled starting materials for the kinetic studies will be discussed.

ID74: High-resolution mapping of IgA receptor interactions by deep mutational scanning

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Immunoglobulin A (IgA) is a specialised subclass of antibody with niche roles in both systemic and mucosal defence. Despite being capable of directing powerful immune responses towards a multitude of threats during infection, IgA is susceptible to evasion by many infectious diseases. These vulnerabilities, in addition to pharmacokinetic problems, have restricted the use of IgA as a therapeutic scaffold for the treatment of disease. Augmented potency, half-life, and efficacy against infectious agents are key to improving the therapeutic utility of IgA. This optimisation can be progressed by mapping the tolerated mutational landscape of IgA against relevant receptors, such as FcαR1 and TRIM21, but requires the deployment of cutting-edge protein engineering techniques.

We have developed an efficient phage display approach for the expression of aglycosylated IgA Fc domains. Correct assembly of IgA Fc has been validated by ELISA and we observe FcαR1 receptor binding despite a lack of Fc glycosylation. To quantify the plasticity of residues involved in this interaction, we designed a 60-site deep mutational scanning (DMS) library consisting of all possible nonsynonymous substitution pairs (35,400 combinations)[1]. To generate this DMS library, we developed a novel, cost-effective method, that integrates degenerate oligonucleotide dimers and multi-part Golden Gate assemblies. Phage panning and next generation sequencing will be employed to deconvolute the relative contribution of individual mutations to IgA receptor binding, with the goal of finding mutations that enhance IgA therapeutic relevance.

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