Microbiology and Infectious Diseases
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Abstract Booklet

In collaboration with the Division of Infectious Diseases, Department of Medicine, University of Toronto, the Division of Infectious Diseases at the Hospital for Sick Children, the Institute for Pandemics and U of T's postgraduate medical and clinical microbiology programs.

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ORAL PRESENTATIONS

TRAINEE DAY - JUNE 10, 2024

UNIVERSITY COLLEGE RM 140
Oral Presentations

Characterizing Human and Viral Proteins in the HIV-1 Envelope Using Novel Methods in Flow Virometry

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The human immunodeficiency virus (HIV) presents a global public health challenge, with estimates of 38 million people worldwide living with HIV in 2021. While highly effective HIV treatments have been developed to prolong the lives of people living with HIV, continued research on cure and vaccine strategies remain critical. The viral envelope glycoprotein (Env) is the sole viral protein present on the surface of virions and is the primary target of vaccine designs. However, human proteins are also abundantly present on the HIV surface and can impact viral phenotypes including homing and infectivity. Thus, developing experimental tools to characterize proteins on the surface of HIV may help identify new antiviral targets or improve vaccine designs. Despite the importance of characterizing viral surface proteins, current techniques available for this purpose do not support high throughput analysis of individual virions, typically only offering semi-quantitative assessments of virus-associated proteins. To address this gap, we adapted flow cytometry, a technique traditionally used for rapid, high sensitivity characterization of single cells, for use on individual virus particles. Using a flow cytometer with nanoscale detection sensitivity, we applied flow cytometry to viruses, termed ‘flow virometry’ (FV), by developing quantitative protocols to stain cellular and viral glycoproteins on the surface of viruses directly in cell culture supernatants. Using FV and complementary techniques, we demonstrated that the virion-incorporated protein PSGL-1 remains functionally active and can facilitate HIV infection through a mechanism of viral capture and transfer to permissive cells, expanding known roles for this restriction factor. Next, we used FV methodology to discover a panel of novel human proteins present on the HIV surface, among which we found CD38 to be the most intriguing. Finally, we used FV as a new tool to study changes in HIV Env conformations, highlighting the use of FV for vaccine and antiviral studies. Taken together, this thesis demonstrates new advances in calibrated FV as a tool to provide sensitive, high throughput characterization of HIV envelope proteins in a quantitative manner. It also showcases the versatility of FV for protein screening, discovery, and for evaluating subtle changes in protein conformation on single virions.
Characterizing Vaccination Gaps in Older Adults Residing in a Complex Continuing Care Hospital

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Objective: As recommended by the National Advisory Committee on Immunizations (NACI), adult vaccinations are a cornerstone of preventative care, especially for older and medically complex patients in complex continuing care (CCC) hospitals. Yet, there is no framework to ensure up-to-date routine adult vaccinations including those against pneumococcal disease, herpes zoster, and tetanus. This study aimed to characterize the vaccination status of patients in a CCC and identify barriers to vaccination.

Methods: A 10-year retrospective review of vaccinations was conducted of patients aged 50 years and older admitted to a 170-bed CCC program at Hennick Bridgepoint Hospital. This included pneumococcal 23-valent polysaccharide, pneumococcal 13-valent, 15-valent, or 20-valent conjugate, Zostavax, Shingrix, and tetanus toxoid-containing vaccines. Institution and provincial electronic medical records (EMRs) were reviewed and patients were interviewed. A cause-and-effect analysis was performed to identify barriers to vaccination and inform a pareto analysis.

Results: 143 patients met inclusion criteria, and all met NACI criteria for vaccination against the three diseases. The average hospital length of stay was 594 days (range 65 to 6931 days). 98.6% (141/143) were unvaccinated against pneumococcal disease, 99.3% (142/143) against herpes zoster, and 98.6% (141/143) were overdue for tetanus vaccination. Both patients who received pneumococcal vaccination were part of a previous vaccine campaign. Ontario publicly funded none for pneumococcal vaccination and only 12.1% (17/142) for Shingrix. Most frequent were the lack of a vaccine administration record, lack of funding, and lack of a standardized process for vaccine assessments.

Conclusions: These results highlight critical gaps in routine adult vaccinations in chronic care settings and has inspired next steps including a formulary review for the addition of vaccines not funded publicly and EMR support for immunization records. However, this also calls for a broader framework to support adult vaccination in chronic care settings.
Cracking open the genetic evolution of vaccine seed influenza viruses propagated in embryonated chicken eggs or mammalian cell culture

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The majority of seasonal influenza vaccines contain viral antigens from viruses propagated in ovo within the embryonated chicken egg (ECE) system. However, in vitro methods of influenza propagation have been developed for vaccine production due to the periodic occurrence of egg-adaptive mutations on the hemagglutinin (HA) viral antigen surface protein.

A previous paper published by our group describes how administering vaccines with between-dose antigen diversity across a population may lead to increased population immunity, ergo greater overall vaccine efficacy. We hypothesize that while in ovo propagation may occasionally select for egg-adaptive mutations, it would also enable a higher number of non-egg-adaptive mutations to persist, leading to higher diversity than in vitro propagation.

To investigate this, we propagated WHO-recommended vaccine seed strains of Influenza A virus from previous flu seasons in ECEs and Madin-Darby canine kidney (MDCK) cells. We then sequenced the output at each passage and analyzed the number of single nucleotide polymorphisms (SNPs) arising in each sample as well as the abundance of those SNPs relative to the original reference nucleotide at that site. We found that after several passages in ovo and in vitro, samples of the same influenza strain which were propagated in ECEs showed numerous SNPs on the HA protein, while samples of the same virus propagated in MDCK cells showed no SNPs.

These preliminary findings suggest that, while they are linked to egg-adaptive mutations, ECE-based vaccine manufacturing methods may lead to more antigenically diverse influenza vaccines than in vitro propagation in MDCK cells.
In 2022, 1.2 million pregnant women were living with Human Immunodeficiency Virus (HIV), with 82% receiving Combination Antiretroviral Therapy (cART). cART use during pregnancy has been successful in preventing vertical transmission of HIV in over 99% of cases; however, drug effects on the developing fetus remain poorly understood. Preliminary data has revealed that pregnant women living with HIV on protease inhibitor-based cART (PI-cART) experience higher rates of adverse birth outcomes associated with elevated estradiol levels, including preterm birth (PTB), small for gestational age (SGA), and low birth weight (LBW). Studies have observed that male offspring of female mice treated with high E2 doses during pregnancy display various abnormalities in the testes, including low testis weight, low germ cell counts, increased germ cell apoptosis, increased incidence of tumors, and decreased fertility. Therefore, excess E2 exposure during gestation, mediated by PI-cART treatment, may initiate long term decreases in testicular size and sperm production in males.

This study investigated whether male mice exposed to PI-cART in utero present symptoms similar to those of excess E2 exposure. Seminiferous tubule size, lumen diameter, spermatogenesis, and Sertoli Cell (SC) counts were measured. SCs, also known as nursemaid cells, are intimately associated with stem cells within the germinal epithelium, providing nutrition, endocrine factors, and establishing a blood-testis barrier. SC counts exhibited a statistically significant increase in PI-cART treated groups when compared to controls. These findings establish a need for further characterization of the effects of prenatal PI-cART exposure on endocrine regulation and reproductive health of the fetus.
Using Predicted Viral Epitopes to Identify Missing Mpox-Specific T Cell Responses in Current Vaccines

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Introduction: In 2022, an outbreak of human monkeypox virus (MPXV) spread around the globe. Modified vaccinia Ankara (MVA) vaccines were administered to reduce transmission. We sought to identify MPXV-specific T cell epitopes eliciting strong responses in MPXV recoverees (MPXV-R) and compare responses in MPXV-Naïve (MPXV-N) people to identify targets for new vaccines.

Methodology: Using the Immune Epitope Database, we selected 46 peptides predicted to be highly immunogenic to T cells. Early MPXV proteins related to DNA replication, virulence/immune evasion were considered. Peripheral blood mononuclear cells (PBMCs) from 15 participants were stimulated with the 46 peptides in enzyme-linked immunosorbent spot (ELISpot) assays to quantify antigen-specific T cell responses by interferon gamma (IFN-g) and granzyme B (GzB) release. We hypothesized that IFN-g and GzB release would be greater in MPXV-R people compared to MPXV-N people in response to stimulation with peptides derived from immediate early MPXV proteins.

Results: 8 (54%) participants are in the MPXV-N group, The 7 (46%) MPXV-R participants recovered from an MPXV infection later than 2022. Of MPXV-N participants, 63% are HIV+ (5/8) and 43% (3/7) of MPXV-R participants are HIV+. All HIV+ participants take combined antiretroviral therapy (cART). All participants are male, and ages range from 24-75 years. For MPXV-R participants, 45 of the 46 peptides showed IFN-g responses exceeding the responses observed in MPXV-N participants (Median of total IFNg responses in MPXV-R vs. MPXV-N = 468 SFC/106 PBMC vs. 111 SFC/106 PBMC). For MPXV-R participants, the total IFN-g responses exceeded total GzB responses for every peptide tested (Median of total IFNg vs. GzB responses per peptide = 468 SFC/106 PBMC vs. 22 SFC/106 PBMC). We observed higher IFN-g responses in HIV+ MPXV-R participants compared to HIV- MPXV-R participants (Median of total IFNg responses in HIV+ vs. HIV- MPXV-R participants = 385 SFC/106 PBMC vs. 51 SFC/106 PBMC). Experiments are ongoing and we are recruiting more participants, especially unvaccinated controls, such that we have enough people to perform more robust statistical analysis.

Conclusion: The peptides we selected induced strong IFN-g inducing responses in MPXV-R participants, while MPXV-N participants showed fewer cytokine responses to MPXV peptides. We observed HIV+ participants have higher IFNg responses to MPXV peptides at baseline Our
results suggest that MVA immunization does not induce strong responses to early and immunogenic T cell epitopes against MPXV which may limit vaccine efficacy.

**Topographical modification of medical gloves to reduce microbial transmission**

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Touch transmission of pathogens remains a significant cause of infectious disease spread within healthcare settings. These infections can be caused by a wide variety of microorganisms, including the continuing rise of antimicrobial resistant species (AROs). A common component of this touch transmission is the interaction of gloved healthcare workers’ hands with infected and non-infected persons, as well as the surfaces within their environments. Although the contamination potential and likelihood of medical gloves has been well documented, few studies have explored the physical characteristics and properties of medical glove surfaces which facilitate transmission. Our research explores the microbial contamination of several medical glove materials (nitrile, latex, vinyl, and isoprene) and the application of microtopographies to the glove surface which can reduce both the contamination and transmission of microorganisms. Through lithography techniques, several types of microtopographies of varying size (2 – 50 micrometers) were successfully molded onto nitrile glove samples. Using a simulated touch contact testing apparatus, microbial attachment, and transmission of several species (Staphylococcus aureus, Staphylococcus epidermidis, Eschericia coli, Klebsiella pneumoniae, Enterococcus faecium, and Candida albicans) was assessed after contact (1N of force for 15 s) with a droplet-contaminated fomite (steel, ABS, or glass) surface. Our results show that a 1.5 -2 log reduction in the contamination of medical gloves against all tested microbes is achieved through the addition of a stable non-wetting topography. This reduction extends to the transfer of microorganisms to a secondary sterile surface, and as a result there should be an additive effect for multiple touch events. Further experiments aimed at assessing the durability of these topographical modifications showed that these reductions can be sustained under larger applied forces (< 9N). These preliminary results highlight the potential that topographical modifications to the medical glove surface can have on the transmission of pathogens through touch contact events.
Vaginal Lactobacillus crispatus persistence following application of a live biotherapeutic product: colonization phenotypes and genital immune impact

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Background: Bacterial vaginosis (BV) increases HIV acquisition risk, potentially by eliciting genital inflammation. After BV treatment the vaginal administration of LACTIN-V, a live biotherapeutic containing the Lactobacillus crispatus strain CTV-05, reduced BV recurrence and vaginal inflammation; however, three months after product cessation CTV-05 colonization was only sustained in 48% of participants.

Results: This nested sub-study in 32 participants receiving LACTIN-V finds that 72% (23/32) demonstrate clinically relevant colonization (CTV-05 absolute abundance > 10⁶ CFU/mL) during at least one visit while 28% (9/32) of women demonstrate colonization resistance, even during product administration. Immediately prior to LACTIN-V administration, the colonization resistant group exhibited elevated vaginal microbiota diversity. During LACTIN-V administration, colonization resistance was associated with elevated vaginal markers of epithelial disruption and reduced chemokines, possibly due to elevated absolute abundance of BV-associated species and reduced L. crispatus. Colonization permissive women were stratified into sustained and transient colonization groups (31% and 41% of participants, respectively) based on CTV-05 colonization after cessation of product administration. These groups also exhibited distinct genital immune profiles during LACTIN-V administration.

Conclusions: The genital immune impact of LACTIN-V may be contingent on the CTV-05 colonization phenotype, which is in turn partially dependent on the success of BV clearance prior to LACTIN-V administration.
Impact of Penile-Vaginal Sex on the Microbiome and Immunology of the Penile Urethra

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Background: The penile urethra is a key HIV acquisition site in heterosexual men. In the penile coronal sulcus and the vagina, inflammation and microbiome composition play an important role in HIV exposure outcome, and both are affected substantially by penile-vaginal sex. However, the direct impact of penile-vaginal sex on these factors in the penile urethra is not known.

Methods: The Sex, Couples and Science (SECS) study was designed to examine the short-term impact of penile-vaginal sex on the genital immunology and microbiome of established couples. First-void urine was collected to characterise the penile urethra microbiome (through 16S sequencing and qPCR) and immune milieu (through a multiplex chemiluminescent immunoassay) before and after sex (immediately, 1, 7 and 72 hours after).

Results: Penile-vaginal sex induced immediate urethral inflammation that resolved within 1 hour. The urethra was also transiently enriched for the common vaginal species Lactobacillus crispatus and jensenii for up to 7 hours. Interestingly, species that are common in the vagina and that may cause vaginal inflammation and increase HIV risk – namely L. iners and Gardnerella vaginalis – were already prevalent in the urethra prior to sex, and remained unaltered. The urethral immune changes seen did not reflect cytokine transfer from vaginal secretions or semen, and were not linked to changes in the microbiome.

Conclusions: Penile-vaginal sex caused transient urethral inflammation and extensive microbiome transfer from the vagina into the urethra, with potential implications for penile HIV susceptibility. However, “non-optimal” vaginal bacteria associated with higher HIV risk in women were already present in the urethra, reflecting long term colonization and suggesting that the urethra is a reservoir for non-optimal vaginal bacteria.
Profilin miRNA changes in Epstein-Barr virus lytic infection identifies a function for BZLF1 in upregulating miRNAs from the DLK1-DIO3 locus

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Epstein-Barr virus is a herpesvirus causing persistent infections in 90% of the global population and is the causative agent of multiple types of cancers and multiple sclerosis. Its success is due in part to its latent and lytic modes of infection. Cellular and viral miRNAs are thought to play important roles in regulating Epstein-Barr virus infection. However, to date, most studies have focused on latent infections in B-cells. To determine how cellular and viral miRNAs contribute to EBV lytic infection in epithelial cells, we conducted miRNA-Seq experiments in EBV-infected AGS gastric carcinoma cells, before and after reactivation to the lytic cycle, analysing both total miRNA and Ago2-associated miRNAs. We identified over 100 miRNAs whose association with Ago2 was affected upon EBV reactivation, most of which were due to changes in miRNA abundance. For EBV miRNAs, the most striking result was that the BHRF1 miRNAs, previously only reported to be expressed in B cells, were upregulated upon reactivation. The largest changes in cellular miRNAs upon EBV reactivation were increases in the abundance and Ago2-association of miR-409-3p, miR-381-3p and miR-370-3p. These miRNAs appear to be pro-viral, as inhibiting all three together reduced EBV lytic protein expression. Interestingly, these miRNAs originate from the IncRNA C14MC within the DLK1-DIO3 locus (GRCh37.p13, 14q32.31). Additional IncRNAs from this locus were also found to increase upon EBV reactivation in nasopharyngeal carcinoma cells lines and occurred very early in the lytic cycle at the time of BZLF1 expression. In keeping with this timing, BZLF1 expression on its own was found to be sufficient to induce MEG8, MEG9 and MIR381HG transcripts. Therefore, we have identified a new role for BZFL1 in inducing the expression of IncRNAs and miRNAs from the DLK1-DIO3 locus, resulting in induction of a subset of encoded miRNAs that promote lytic infection.
Background: Gay, bisexual, and other men who have sex with men (GBM) have a high burden of human papillomavirus (HPV)-associated diseases and thus are prioritized for HPV vaccination. Since 2015/16, several Canadian provinces have offered publicly funded HPV vaccination for GBM aged ≤26 years. We evaluated the uptake and effectiveness of HPV vaccine in GBM soon after these targeted HPV immunization programs were implemented.

Methods: The Engage Cohort Study is a prospective cohort of 2,449 GBM aged ≥16 years in Vancouver, Toronto, and Montreal. Participants were recruited using respondent-driven sampling from 2017 to 2019 and followed for 12 months. At each visit, participants self-reported their HPV vaccination history and, for a subset of younger GBM aged 16-30 years, self-collected anal specimens for HPV DNA testing.

Results: Over one-quarter of Engage participants were infected with ≥1 vaccine-preventable type at baseline. HPV vaccination (self-reported receipt of ≥1 dose) was associated with lower anal HPV prevalence, incidence, and persistence of vaccine-preventable types, independent of sexual risk behaviours and after correcting for non-differential exposure misclassification associated with self-reported HPV vaccination.
Vaccine effectiveness was higher when vaccination was initiated at younger ages or soon after sexual debut. HPV vaccine coverage increased over the follow-up period, with higher uptake among younger GBM who were eligible for the publicly funded programs (60-73% across cities at 12 months).

Conclusions: Despite targeted HPV immunization programs, anal HPV infection was highly prevalent among young, sexually active Canadian GBM. As of 2021, HPV vaccine coverage remained below the national target of 90%, suggesting that many GBM remain at risk for HPV and its associated diseases. Community-based strategies are needed to improve HPV vaccine access and uptake through targeted immunization programs, alongside school-based universal programs. These findings will inform best practices for HPV primary prevention in GBM.
Investigating the role of a bacterial heme acquisition system in macrophage persistence

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Homeostasis and the acquisition of essential micronutrients, such as heme, is an important area of coevolution between the host and bacterial pathogens. The host sequesters the micronutrients to starve pathogens; however, bacteria also have specialized nutrient acquisition systems. Our lab discovered a bacterial heme acquisition system in Acinetobacter baumannii, consisting of transporter SLAM which secretes HphA to steal heme from host hemoglobin for delivery to the bacteria via its receptor, HphR. This system is conserved across diverse Gram-negative bacteria, including human pathogen Stenotrophomonas maltophilia. S. maltophilia's HphA has a non-canonical role in promoting bacterial persistence in macrophages by upregulating anti-inflammatory cytokine, IL-10. My project’s objective is to systematically explore if the macrophage persistence role is conserved across HphAs from diverse species. To test this objectives, I reconstituted the SLAM-HphA from S. maltophilia, A. baumannii and Haemophilus haemolyticus into a lab Escherichia coli strain. Then, I infect mouse macrophages with the HphA-secreting or a SLAM-only E. coli strains and assess the intracellular bacterial survival and macrophage cytokine profiles post-infection. We have shown significantly improved intracellular survival of all HphA-secreting strains relative to the SLAM-only strain. Additionally, all the HphA-secreting strains trigger an IL-10 upregulation relative to the SLAM-only strain. This suggests that like HphA’s heme acquisition role, its macrophage persistence role is also conserved across diverse species. Overall, this study will provide novel insights into host-pathogen interactions and potential antimicrobial targets.
Leveraging MNK3 cells to study group 3 innate lymphoid cell responses to infectious challenges

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Group 3 innate lymphoid cells (ILC3) are critical for tissue homeostasis and host defense in the gut. They respond to signals in the local microenvironment and serve as an important source of the cytokines interleukin (IL)-22 and Colony Stimulating Factor 2 (CSF2), both of which help protect the intestine by driving epithelial barrier integrity and myeloid cell function. Due to the technical challenges of isolating and manipulating primary ILC3s, a novel cell line was developed in 2015, named MNK3 cells. These cells phenocopy primary ILC3s functionally and transcriptionally, making them an attractive tool for in vitro manipulation. We sought to investigate whether CRISPR/Cas9-mediated gene editing is feasible in MNK3 cells to delete ILC3-related genes. Furthermore, we reasoned that adoptive transfer of gene edited MNK3 cells into mice would serve as useful approach to investigate the role of key genes in ILC3 functions in vivo. We first established successful protocols for gene editing and adoptive transfer of MNK3 cells into lymphopenic Rag2−/-Il2rg−/- mice. We observed reliable reconstitution of MNK3 in the intestine and validated that MNK3 cells execute central functions of primary ILC3s in vivo. Moreover, in the context of infection with the enteric pathogen Citrobacter rodentium, engrafted MNK3 cells confer protection against systemic dissemination. Using our system, we establish that gene editing and reconstitution of lymphopenic mice with MNK3 cells are a suitable model to investigate the role of ILC3 functions in vivo, enabling us to further investigate genes with unknown functions in a central immune cell type that are crucial in protection against enteric infections.
POSTER PRESENTATIONS

MAIN PROGRAM - JUNE 11, 2024

MEDICAL SCIENCES BUILDING,
DAVID NAYLOR STUDENT COMMONS

NOTE
EVEN NUMBERS ARE IN SECTION A.
ODD NUMBERS ARE IN SECTION B.
Background: An infant PCV13 program (2+1) was introduced in Ontario in December 2010. We assessed the epidemiology of IPD in children in the late post-PCV13 era.

Methods: We perform population-based surveillance for IPD (pop’n 4.5M). Microbiology labs report sterile site isolates of pneumococci; annual audits ensure completeness. The National Microbiology Laboratory serotypes isolates. Statistics Canada provides population data. Clinical data are from chart review and patient/MD interview. Complete vaccination is defined per NACI. Cases of vaccine serotype (ST) disease are categorized as: ineligible for vaccination, vaccine failure (completely vaccinated); program failure (un- or in-completely vaccinated); partially vaccinated (vaccination up-to-date but incomplete).

Results: In the nine years from 1/1/2014 to 31/12/2022, 373 IPD episodes were identified among children (<15 years), with 270 (70%) in children 0-4 years old. Clinical data were available for 349 (94%), and serotyping for 358 (96%). 124/349 (36%) children had an underlying illness predisposing to IPD, and 65 (19%) were immunocompromised (Table 3). Underlying conditions were more common among 5-14 yrs than 0-4 year-olds: any condition 48% vs 31%, P=.002, immunocompromising illness 37% vs 12%, P<.0001. IPD incidence in 2022/2023 was 12.8/100000/year among <5y-olds, 2.5/100000/year among 5-17y-olds, not different from incidence in 2014-2019 (Figure). Overall, 84/358 cases (23%) were PCV13 STs, 47 (13%) PCV15/not13, 97 (27%) PCV20/not15, 130 (36%) were non-PCV. PCV13 ST were: 41 ST 19A (49%), 32 (38%) ST 3, 6 (7%) 19F, 3(3.5%) 7F, 1 each 14, 9V. Of 78 (88%) PCV13 IPD with evaluable vaccine history, 25 were not eligible (5 <2mos, 20 too old to have received PCV13), 33 were vaccine failures, 16 were program failures (9 unvaccinated, 7 no >12m dose); 5 incompletely vaccinated.

Conclusions: Post PCV13 implementation, IPD has stabilized, with increased disease due to PCV15 and PCV20 STs; some PCV13 disease persists. Higher-valency vaccines should significantly reduce IPD, a “catch-up” dose might be considered for immunocompromised older children.
Leishmania-Induced Dendritic Cell Migration and Its Potential Contribution to Parasite Dissemination


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Leishmania, an intracellular parasite species, causes lesions on the skin and in the mucosa and internal organs. The dissemination of infected host cells containing Leishmania is crucial to parasite survival and the establishment of infection. Migratory phenomena and the mechanisms underlying the dissemination of Leishmania-infected human dendritic cells (hDCs) remain poorly understood. The present study aimed to investigate differences among factors involved in hDC migration by comparing infection with visceral leishmaniasis (VL) induced by Leishmania infantum with diverse clinical forms of tegumentary leishmaniasis (TL) induced by Leishmania braziliensis or Leishmania amazonensis. Following the infection of hDCs by isolates obtained from patients with different clinical forms of Leishmania, the formation of adhesion complexes, actin polymerization, and CCR7 expression were evaluated. We observed increased hDC migration following infection with isolates of L. infantum (VL), as well as disseminated (DL) and diffuse (DCL) forms of cutaneous leishmaniasis (CL) caused by L. braziliensis and L. amazonensis, respectively. Increased expression of proteins involved in adhesion complex formation and actin polymerization, as well as higher CCR7 expression, were seen in hDCs infected with L. infantum, DL and DCL isolates. Together, our results suggest that hDCs play an important role in the dissemination of Leishmania parasites in the vertebrate host.
Host protein incorporation by HIV-1: new mechanisms for immune modulation.

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Background: HIV-1 buds through cellular membranes to acquire its outer lipid envelope. In doing so, it becomes imprinted with human proteins from the cell membrane that can impart additional functional properties to virions. Herein, we demonstrate the utility of flow virometry (FVM) techniques to characterize the impact of virion-incorporated proteins, with emphasis on the myeloid antigen, CD14.

Findings: We coupled our FVM techniques with BioLegend’s LegendScreen antibody panel to interrogate the presence of 360 human antigens on the HIV surface, revealing 59 novel incorporated proteins and highlighting the utility of the technique in fingerprinting virions. Next, we showed that lab-adapted isolates and pseudoviruses of HIV-1 incorporate CD14 into their viral envelope. This finding proved significant as CD14 on virions was able to bind bacterial lipopolysaccharide (LPS) with high efficiency. Moreover, we showed that virions with incorporated CD14 were able to bind and transfer LPS to TLR4-reporter cells and THP-1 monocytes, resulting in NF-κB and IRF-3 activation, and secretion of pro-inflammatory cytokines TNF-α and CCL5.

Significance. Our data demonstrates the utility of FVM for performing protein screens on virion surfaces that can characterize a protein fingerprint of virions and potentially identify the tissue source, or reservoir, of virus producer cells. For example, CD14 is an incorporated myeloid antigen typically expressed on macrophages, a target cell of HIV-1. Our CD14 studies also highlight a new potential for virion-incorporated human proteins to facilitate shuttling of bioactive molecules and triggering of inflammatory responses by immune cells, offering novel insights into viral pathogenesis.
Novel roles of the Epstein-Barr virus BMRF1 protein and the host NuRD complex in Epstein-Barr virus infection.

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Epstein-Barr virus (EBV) is a ubiquitous herpesvirus that infects ~90% of the human population with interplay between latent and lytic cycles of replication. EBV is also the causative agent for multiple malignancies, as well as infectious mononucleosis and multiple sclerosis. BMRF1 is an essential viral replication protein that acts as the viral DNA polymerase processivity factor and localizes to sites of replicating EBV DNA (replication centres) during lytic infection. BMRF1 has additional roles in activating the transcription of some EBV late genes. To characterize BMRF1’s interaction with cellular proteins, our lab performed affinity purification coupled to mass spectrometry, and found that BMRF1 interacts with the cellular NuRD (nucleosome remodelling and deacetylation) complex, which affects cellular gene expression and DNA repair through chromatin modifications. We have determined that BMRF1 interacts with the cellular NuRD (nucleosome remodelling and deacetylation) complex, which affects cellular gene expression and DNA repair through chromatin modifications. We have determined that BMRF1 binds the MTA2 and RBBP4 subunits of NuRD through a consensus motif, and that point mutation of this motif in BMRF1 abrogates NuRD binding and transcriptional activation by BMRF1. In addition, NuRD subunits co-localize with BMRF1 to EBV replication centers, and silencing NuRD subunits results in decreased EBV genome amplification during lytic infection, with negative effects on late gene expression. The results suggest that BMRF1 uses NuRD for transcriptional activation and that NuRD also plays a role in EBV genome replication.
A Rise in the Frequency of Carbapenemase-producing Enterobacterales Following an Initial Decline Post COVID-19

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OBJECTIVES: Carbapenemase-producing Enterobacterales (CPE) are a major health threat. Introduction of CPE is facilitated by international travel from areas of high prevalence. Global travel dramatically reduced during the COVID-19 pandemic. CPE numbers in a tertiary-care clinical microbiology laboratory servicing a metropolitan urban region decreased during this time period and remained low through to 2022. The aim of this study was to review changes in the frequency of CPE at this site in 2023.

METHODS: The total number of CPE per year (all CPE per year excluding duplicates) and the total number of individual carbapenemases per year (all carbapenemase per year excluding duplicates) per 1,000,000 bacteriology clinical and CPE screening cultures were graphed from 2009 through 2023. The trend from 2009 through 2019 (pre-COVID-19) was compared to that from 2019 through 2023. Chi-squared test for trend was completed for each time period using GraphPad Instat.

RESULTS: Prior to 2019, the total number of CPEs isolates per 1,000,000 bacteriology clinical and CPE screening cultures rose steadily from 5 (2009) to 130 (2014) and 172 (2019) (P<0.0001). During the pandemic, CPE isolates per 1,000,000 cultures declined from 2019 to 2022, from 172 to 74 (P=0.004). In 2023, CPE isolates increased considerably to 150 per 1,000,000 cultures. The recent increase in CPE correlated with increased frequency of NDM and OXA-48 carbapenemases-producing organisms.

CONCLUSIONS: The frequency of CPE detection, which declined in the three years after the COVID-19 pandemic, has returned to near pre-pandemic amounts. The return of global flight travel to nearly pre-pandemic volumes and the ongoing reduction of use of COVID-19 public health measures in the community correlated with this rise in identified CPE.
Donor diversity in fecal microbiota transplantation: microbial composition differences in donor stool microbiomes

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Fecal microbiota transplantation (FMT) is a treatment option for recurrent Clostridiodes difficile infection. A FMT program in an urban metropolitan region performs rigorous clinical and microbiology laboratory-based screening tests on stool donors. Age, BMI, medication history, high-risk activity, and physical and mental health are assessed within eligibility criteria. The aims of this work were to determine the microbial diversity in donor stool from individuals who passed all FMT donor screening tests and to determine C. difficile recurrence rates in recipients who received FMTs from donor stool as therapy for recurrent C. difficile infection.

Three stool donations from five stool donors (Female=2, Male=3) were collected (n=15), each 2-4 months apart (average= 3 months (1st-2nd donation) and 2.8 months (2nd-3rd donation)), and processed for 16S rRNA gene sequencing. Genus level relative abundance, alpha and beta diversity were determined. The proportions of recipients who did not acquire a C. difficile infection within a 3 month period post-FMT series were used to calculate success rates of donor stool donations.

Relative abundance profiles were similar between Donors 1 and 2, and Donors 3 and 4. Donor 5 had a dissimilar profile with a significantly higher abundance of Prevotella spp. Success rates of donor stool were variable: 67% (14/21) for Donor 1, 75% for Donors 2 (6/8) and 3 (6/8), 92% (22/24) for Donor 4. Donor 4 had both the highest alpha diversity indices and the greatest success rate in FMT recipients.

Although stool donors are subjected to the same eligibility criteria and screening tests to donate in a FMT program, the composition of stool microbiomes significantly differ across donors with different success rates of donor stool. Stool compositional differences may be attributed to early childhood exposures, epigenetics, donor diet, and lifestyle influences. FMT programs should consider incorporating additional variables in their donor screening criteria.
Objectives: Annual antibiograms serve as a guide for empirical therapy. However, susceptibility is presented as an average percent susceptible, providing only a single static interpretation of susceptibility. To detect emerging antimicrobial resistance, especially those that are subtle, graphing susceptibility over time can visualize trends. The purpose of this study was to analyze susceptibility trends in the most prevalent organisms in a single tertiary-care academic hospital over time.

Method: Antibiotic susceptibility of bacterial isolates of blood and urine samples from 2013 to 2021 of a tertiary-care academic hospital was obtained from annual antibiograms created following the Clinical and Laboratory Standards Institute (CLSI) M39 document. Updated CLSI M100 breakpoints were used for all antibiograms throughout the study period. Trends were shown using Excel.

Results: Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa were the most prevalent Gram-negative organisms from blood and urine cultures. Susceptibility trends for E. coli and K. pneumoniae are shown in Figure 1. E. coli displayed a subtle trend in decreasing susceptibility for ceftriaxone, and a similar trend is seen for ceftriaxone, trimethoprim-sulfamethoxazole and tobramycin in K. pneumoniae. From 2019, urine isolates show a notable reduction of 38% in tobramycin susceptibility in E. coli and 78% reduction in K. pneumoniae. Not shown in Figure 1, blood and urine P. aeruginosa and blood and urine Staphylococcus aureus and enterococci, the most prevalent Gram-positive organisms, showed relative stability in susceptibility.

Conclusion: Reporting antimicrobial susceptibility over time for bacterial isolates, as opposed to only reviewing static data at a single point of time, is valuable for detecting emerging resistance. Subtle trends, such as those seen for ceftriaxone from blood Gram-negative isolates, and dramatic trends, such as those seen with tobramycin from urine Gram-negative isolates, can go unnoticed by review of mass data sets presented in separate disconnected annual antibiograms.
Enhancing Antimicrobial Stewardship Through Generating Double Drug Antibiograms

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Objective: Broad spectrum empiric antimicrobial therapy is crucial for patients presenting with febrile neutropenia. Antibiograms typically provide susceptibility coverage for single drugs. The study analyzed antimicrobial susceptibility data for double drug combinations and compared them to local hospital guidelines for treatment of febrile neutropenia.

Method: Susceptibility data for 2023 blood culture isolates from a cancer hospital serving a large urban area were presented in an antibiogram following 2022 CLSI M100/M39 guidelines. Susceptibility to piperacillin-tazobactam and tobramycin, meropenem and tobramycin, ceftazidime and tobramycin, piperacillin-tazobactam and ciprofloxacin, meropenem and ciprofloxacin, and ceftazidime and ciprofloxacin combinations were compared against recommendations for empiric treatment from local hospital-based guidelines.

Result: Gram-negative isolates had an associated 65% weighted average susceptibility to piperacillin-tazobactam. When paired with tobramycin or ciprofloxacin, the percent susceptibility rose to 91% and 88% respectively. A similar increase was seen for ceftazidime, where there was 71% susceptibility to the drug alone which increased to 91% and 88% when paired with tobramycin and ciprofloxacin, respectively. Percent susceptibility for meropenem stayed relatively stable at 97% when considered alone or when paired with tobramycin, and 97% when paired with ciprofloxacin. Gram-positive isolates did not show changes in susceptibility with the combinations studied. Local hospital febrile neutropenia hospital guidelines vary with one hospital recommending treating with piperacillin-tazobactam alone and another recommending treatment with a combination of piperacillin-tazobactam with tobramycin or with meropenem alone.

Conclusion: For gram-negative blood isolates from cancer hospital patients, the efficacy of piperacillin-tazobactam and ceftazidime is increased when paired with tobramycin or ciprofloxacin. Meropenem alone demonstrated high percentage of susceptibility. Centres suggesting the use of piperacillin-tazobactam alone should recognize the limitations of its coverage and the added coverage provided by combination treatment. It is valuable for institutions to incorporate combination drug susceptibility data into their antibiograms to help guide empiric recommended treatments.
**InlC of Listeria monocytogenes exploits the tumor suppressor, CYLD, to regulate bacterial spread.**

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Listeria monocytogenes (Lm) is a bacterial pathogen ubiquitously found in the environment, and consequently, a prominent contaminant in the food industry. Once ingested, Listeria adopts an intracellular lifecycle and utilizes a plethora of virulence factors to discreetly persist and spread throughout the body, causing an often-lethal form of meningitis in the immunocompromised and elderly. As the function of many Lm proteins are poorly understood, here we harnessed the versatility of biotin proximity labelling (BioID) to characterize the interactome of Internalin C; a Lm effector that is robustly secreted into the host cell upon infection. Despite its small size (25 kDa), we observed InlC facilitates multiple, concurrent interactions with immune-related complexes to help fine-tune the intracellular landscape for pathogenesis. Mass spectrometry revealed the host deubiquitinase, CYLD, as a top interactor corroborated by subsequent protein crystallography and biochemical analyses. In the context of macrophages who are first-line defenders in the liver, we observed that InlC regulates Lm cell-cell spread in a CYLD-dependent manner both in vitro and in vivo. Collectively, this work demonstrates the potential of proximity labelling to better understand the molecular interplay between microbial species and their hosts, with applications including, but not limited to, human disease.
Defense against a Legionella pneumophila phage is linked to increased virulence in the accidental human host

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The gram-negative bacterium Legionella pneumophila is an accidental and opportunistic pathogen of human alveolar macrophages, resulting in a severe form of pneumonia known as Legionnaires’ disease. L. pneumophila transmission is purely environmental and normally cannot spread from person to person—the primary mode of acquisition being the inhalation of contaminated aerosols. Therefore, all evolutionary pressures are environmental in nature, and selection for infectivity in the human host must be influenced by the bacterium’s regular life cycle, including encounters with its preferred amoebae hosts or potential parasites, such as bacteriophages. However, to date, no phage has been identified for the Legionella genus. Utilizing L. pneumophila’s CRISPR-Cas system as a record of previous infections, we identified a 30-kb integrative element, Legionella mobile element-1 (LME-1), that is being actively defended against and has distant homology to structural phage proteins. LME-1 is unamenable to traditional phage induction methods; thus, we developed a genetic approach to produce phage particles that can be purified by cesium chloride gradient centrifugation. Characterization through electron microscopy, mass spectrometry, and Illumina sequencing of purified particles corroborate that LME-1 particles contain a complete genome with all the necessary structural components for phage assembly. LME-1 particles are infectious to specific strains of L. pneumophila; and through isolation of spontaneous susceptible mutants, we identified a key LME-1 resistance factor: the LPS O-chain acetyltransferase Lag-1. Lag-1 confers protection to L. pneumophila during the attachment stage of LME-1’s infectivity cycle, likely by obstructing access to an LPS O-antigen receptor—a common target for phage attachment. Furthermore, lag-1 has been identified as one of the major determinants of virulence in humans by conferring resistance to complement-mediated killing (Wee et al. 2021, Nat Comm), placing LME-1 as an environmental selective pressure that selects for virulence in humans via the acquisition and maintenance of lag-1.
Whole Genome Sequencing for the Identification of a Streptococcus agalactiae Outbreak in Neonatal Intensive Care Unit

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Background: Group B Streptococcus (GBS), typically transmitted to infants from mothers, can also be acquired in healthcare settings like neonatal intensive care units (NICUs). Whole genome sequencing (WGS) is useful for investigating NICU GBS outbreaks.

Objective: To investigate NICU clusters of GBS infections using WGS.

Methods: Mount Sinai Hospital's infection prevention and control (IPAC) team monitors late-onset GBS disease (LOD) in 57-bed NICU. An investigation began when two LOD GBS cases occurred within two weeks. WGS was done on isolates from initial and subsequent cases, and past invasive GBS cases over six months. WGS was conducted using Illumina at Canada's National Microbiology Laboratory. Patient charts were reviewed for shared exposures.

Outbreak Description and Investigation: In August 2023, two neonates had LOD GBS disease two weeks apart. Two more cases were identified during the wait for WGS results. Isolates from three of four patients were identical, including one from an earlier admitted patient with GBS bacteremia on their first day (first cluster). Weekly point prevalence checks over three weeks identified five infants with unrelated strains. Another infant developed GBS conjunctivitis from a different strain, matching the fourth patient (second cluster). Monitoring discontinued after three consecutive negative weekly point prevalence results. Three months later, two more cases detected: one with bacteremia and the other with asymptomatic bacteriuria. WGS showed matching isolates (third cluster).

IPAC Interventions: Observations revealed IPAC lapses, with no commonalities among cases except unit proximity. Foundational IPAC measures were reinforced, but no additional precautions were implemented due to private room structures. No environmental samples were taken due to multiple strains and an unclear source.

Conclusions: Awareness of healthcare-associated transmission is critical in NICUs as LOD GBS cases emerge. GBS outbreaks can involve multiple strains and last long. WGS is vital for confirming transmission and guiding investigations.
Characteristics of resurgent invasive group A streptococcal infection post-pandemic in Toronto, Canada


Background and objectives: In the wake of the COVID-19 pandemic, global invasive group A streptococcal (iGAS) infections surged. In Ontario, Canada, the resurgence began in late 2022. We evaluated iGAS infection epidemiology during 2022 and 2023, comparing it to pre-pandemic data (2011-2013).

Methods: Since 1992, the Toronto Invasive Bacterial Diseases Network has conducted population-based surveillance for iGAS in Toronto/Peel Region, Ontario. Laboratories serving residents report GAS isolates from sterile sites to a central study office. emm typing is provided by Canada's National Microbiology Laboratory.

Results: 553 iGAS cases were identified in 2022/23, 10% (56) in children (<18y). Median age was 54.1y (IQR 35.8-69.9); 64% were male. Incidence increased from 2.2 per 100,000 in 1992 to 6.2 per 100,000 in 2019, then declined in 2021/22 before surging to 10.2 cases/100,000 in 2023. The most common presentation was soft tissue infection: 36% in children and 55% in adults. In children, pneumonia (23%) was also common. Overall, 63% of all patients had comorbidities; and 19% of the adults were homeless.

Blood cultures were positive in 415/511 (81%) cases. In children, the most common emm types were 1 (49%) and 12 (31%); in adults, the most common were 12 (14%), 49 (14%), 1 (13%), and 82 (12%). M1UK comprised 62.4% of emm1 cases. Streptococcal Toxic shock syndrome (STSS) occurred in 13% and necrotizing fasciitis (NF) in 7.1%, with no difference in proportion between adults and children or between the three time periods. The case fatality rate (CFR) was 12%. M1UK infections were not associated with STSS, NF or death. Pneumonia was more common pre-pandemic and in 2023 compared to 2022 [p 0.01 and 0.03 respectively].

Conclusions: iGAS has resurfaced in Canada, displaying distinct characteristics in pediatric and adult patients. Ongoing regional surveillance aids in monitoring incidence, identifying at-risk populations, and tracking emm-type distribution.
Lyme disease, caused by the bacteria Borrelia burgdorferi in North America and primarily transmitted by deer ticks, has symptoms such as fever, and the characteristic erythema migrans rash.(1,2) If untreated, it can lead to complications affecting the nervous and cardiac systems. Ticks also serve as vectors for the parasite Babesiosis and Powassan Virus. With the rise in tick populations due to climate change, incidences of tick-borne diseases are also increasing.(1,2)

Diagnosing Lyme disease usually involves examining patients' tick history, symptoms, and a serology test.(1,2) Early target detection is critical for effective antibiotic treatment. However, its slow onset, along with common symptoms overlapping with other diseases make diagnosis difficult. (1,2) Additionally, current tests are laboratory-based, requiring trained scientists and costly resources. This is exacerbated in low-income countries, highlighting the need for more accessible testing.(3)

We propose to use a new CRISPR-Cas12a based system developed in our lab. Upon target detection, CRISPR-Cas12a will cut the target, activating its nonspecific cleavage activity(4). It can then interact with our nanoparticle reporter system. To ensure clinically relevant detection by the CRISPR-Cas12a system, loop-mediated isothermal amplification (LAMP)(5) is introduced upstream. These reactions will take place in tubes within a 3D-printed box(6).

Citations:
Identifying pathogenic-related traits of Gardnerella in bacterial vaginosis

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The vaginal microbiome of reproductive aged women is dominated by Lactobacillus bacteria. Bacterial vaginosis (BV) is the disruption of the normal vaginal flora characterized by an overgrowth of anaerobic non-Lactobacillus bacteria. BV symptoms include malodour, itching and abnormal vaginal discharge. Gardnerella is strongly associated with BV, as their population in the vaginal microbiome is significantly increased in women with BV versus healthy women. During BV, Gardnerella outcompete Lactobacillus, forming thick biofilms on vaginal epithelial cells. Interestingly, some women with Gardnerella-abundant vaginal microbiomes are asymptomatic for BV. Based on this observation it is hypothesized that Gardnerella pathogenicity is species or sub-group dependent. The Gardnerella genus of bacteria is genetically diverse; subdivided into 4 main sub-groups (clades). This project aims to investigate Gardnerella can be divided into pathogenic and non-pathogenic groups. To conduct these studies, a collection of Gardnerella bacteria from vaginal swabs of Kenyan women of varying BV statuses (negative, intermediate, or positive) have been isolated and cultured. The Gardnerella isolates have been designated into their respective clades and species using PCR-based methods. The current collection of Gardnerella isolates include at least one isolate for all 4 clades; this will be expanded as isolations from vaginal swabs continue. After unique Gardnerella strains from the isolate collection have been identified, specific phenotypes related to BV pathogenesis will be assayed to determine associations between Gardnerella groups/species/strains and BV status. These phenotypes will include expression of hydrolytic enzymes, biofilm formation, and pili sequence and expression. Additionally, co-culture experiments aimed at identifying isolates that can outcompete Lactobacillus under varying conditions relevant to the vaginal environment will be conducted.
Humans share a core fecal miRNome potentially targeting probiotic Lactobacillus genes

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Background: Host gut microbiome crosstalk is essential in maintaining gut homeostasis and host microRNAs (miRNAs) have emerged as an important player. MiRNAs in the intestinal content (fecal miRNAs) have been proposed as biomarkers of disease and as modulators of bacterial growth upon entering bacterial cells. Interestingly, few studies show that miRNA can also affect select probiotics, including the largely used lactobacilli. Though, the degree of similarity of the fecal miRNome across individuals is unknown and its potential to target genes in Lactobacillus is under-investigated.

Aims: To determine the shared fecal miRNA signature of healthy individuals and investigate in silico their potential gene targets in the probiotics Lacticaseibacillus rhamnosus R0011 and Lactobacillus helveticus R0052.

Methods: We extracted datasets from published healthy adult human fecal miRNA profiling studies (PubMed and Cochrane) and identified shared miRNA species via UpSet plots in R. Probiotic target of select miRNAs were identified via BLAST and RNAup (ViennaRNA package, for RNA-RNA secondary structure assessment) and filtering total free binding energy ($\Delta G_{\text{total}} < -12$) of the miRNA-gene duplexes. Functions of target genes were obtained from the Clusters of Orthologous Genes (COG) database.

Results: We identified 229 published primary research papers and 7 datasets from 517 individuals were extracted. Hsa-miR-21-5p and hsa-miR-1246 were shared across all samples. 5 and 3 gene targets, respectively, were found in L. helveticus R0052, and 7 and 30 targets were found in L. rhamnosus R0011 for these miRNAs. These targets were found to be involved in various bacterial functions including carbohydrate/amino acid metabolism and transcription.

Conclusions: MicroRNAs commonly found in human feces could potentially affect probiotic growth. Our findings provide insight into host-bacteria relationships in the gut and mechanism underlying probiotic effects.
Characterization of laboratory confirmed Creutzfeldt - Jakob disease from three Ontario Tertiary Care Centers between 2012-2022

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This study characterizes laboratory-confirmed Creutzfeldt-Jakob disease (CJD) cases over a ten-year period from three tertiary hospitals in Ontario, Canada, aiming to address the lack of recent Canadian data on CJD. Retrospective chart reviews of patients diagnosed between 2012-2022 were conducted on 30 patients, focusing on descriptive epidemiology, symptom presentation, and potential missed opportunities. Patients made a mean of 2.2 visits (range 1-5) prior to admission for CJD testing. Common symptom presentations included loss of coordination (63.3%), changes in behavior (60%), progressive mobility loss (53.4%), memory loss (50.0%) and involuntary movements (50.0%). MRI findings showed potential indicators of CJD in 76.7% of cases, including diffuse restriction and asymmetrical hyperintensities. The mean duration from symptom onset to testing was 91 days. Endpoint Quaking Induced Conversion (RT-QuIC) diagnostic testing from cerebral spinal fluid yielded positive results in 90.0% of patients, while 83.3% were positive for 14-3-3 ELISA. Sporadic CJD accounted for 93.3% of cases. Following diagnosis, 46.7% of patients were discharged home, 36.7% were transferred to palliative care, 3.3% to a hospice program, and 36.7% died. The mean time from symptom onset to death was 121 days, and from diagnosis to death was 35 days. A notable increase in CJD diagnoses occurred post-pandemic, with 12 cases in 2022 alone. This study emphasizes the importance of considering CJD as a differential diagnosis and subsequent laboratory testing in the context of appropriate neurologic symptoms that overlap with many non-infectious diseases. This is especially important as most cases were sporadic CJD, and thus patients lacked risk factors and obvious indications for testing.
Currently, one of the most detrimental fungal pathogens to human health is Cryptococcus neoformans. Despite its devastating impact, only the azole and polyene classes of antifungal drugs can be used as single agents to treat cryptococcal disease. Thus, there is a clear need to develop novel antifungals to treat C. neoformans infections. A promising antifungal target is the molecular chaperone Hsp90. Despite its important role in enabling virulence and drug resistance in diverse fungal pathogens, in the setting of infection, the inherent toxicity of inhibiting host Hsp90 necessitates the development of fungal-selective compounds. To explore targeting Hsp90 as a strategy to combat C. neoformans infections, this work leverages a structure-guided approach to develop and characterize fungal-selective Hsp90 inhibitors. Specifically, 51 novel Hsp90 inhibitors were synthesized based on a resorcylate aminopyrazole (RAP) scaffold and tested for fungal selectivity, target engagement, and whole-cell activity. A fluorescence polarization (FP) assay was used to determine the target engagement and selectivity of the compounds towards C. neoformans and human Hsp90 from cellular lysates. Results from this assay indicated that 48 of the 51 compounds had optimal target engagement against fungal Hsp90 (EC50 < 12.5 µM) with minimal target engagement against human Hsp90 (EC50 > 25 µM), resulting in excellent selectivity. Next, a dose-response assay was used to determine if these compounds had whole-cell activity against C. neoformans. Unfortunately, none of the compounds were efficacious against wild-type C. neoformans (MIC80 > 25 µM). Conversely, 11 of the 51 compounds displayed moderate bioactivity against an efflux-compromised C. neoformans strain (MIC80 < 25 µM). These findings highlight the need for additional work to develop fungal-selective compounds with activity against fungal cells. Overall, this work aims to uncover new therapeutic strategies to combat C. neoformans infections by targeting a key regulator of fungal growth and virulence.
Screening the RIKEN Natural Product Depository (NPDepo) Library Identifies NPD8193 as a Broad-Spectrum Antifungal

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Billions of people are infected by fungal pathogens every year, resulting in 3.8 million deaths worldwide. Species from the genera Candida, Aspergillus, and Cryptococcus are the causative agents for over 90% of invasive fungal infections. Treatment of these maladies is complicated by the emergence of drug-resistant isolates and the limited arsenal of antifungals. Thus, novel strategies to combat these fungal infections are needed. A promising approach to identify compounds with antifungal activity is to perform high-throughput compound screens. A subset of the RIKEN Natural Product Depository (NPDepo) library was screened to identify compounds with activity against Candida albicans, Candida auris, Candida glabrata, and Cryptococcus neoformans. This screen identified twenty compounds with activity against all four fungal pathogens, of which three unique compounds were prioritized. In this project, I will characterize the activity of one of the prioritized compounds, NPD8193, and uncover its mechanism of action. To explore the mode of action of NPD8193, we employed haploinsufficiency profiling (HIP), a technique that uses a library of heterozygous deletion mutants to generate predictions as to the compound’s cellular target. The C. albicans HIP data revealed heterozygous deletion of TIP1, a gene that encodes a protein predicted to be involved in biogenesis of endoplasmic reticulum (ER)-derived COPII transport vesicles, as hypersensitive to NPD8193. Haploid deletion profiling (HAP) in S. cerevisiae was also performed, a technique that employs haploid deletion mutants to look for compound hypersensitivity. HAP analysis identified genes involved in the unfolded protein response (IRE1 and HAC1), the ribosome (RPL9B), and endosome-Golgi trafficking (TRS85) as those that confer hypersensitivity to NPD8193. Overall, the chemogenomic profiles suggest a model where NPD8193 inhibits ER-to-Golgi trafficking, inducing cellular stress that relies on the UPR for cell survival. Future work will focus on testing this hypothesis with genetic, biochemical, and cell biological assays.
Defining the allosteric activation path for ClpP

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Molecular chaperones and proteases exist in all organisms where they play a critical role in maintaining cellular protein homeostasis. ClpP is one such protease present in both bacteria and eukaryotes. It is composed of fourteen identical subunits that typically assemble as stacked heptameric rings to form a hollow barrel-like structure with 7-fold symmetry. Chemical interference may be used to activate ClpP and dysregulate its function, resulting in the unregulated proteolysis of non-substrate proteins, causing cell death. As such, targeting ClpP has recently emerged as a promising avenue for the development of novel antimicrobial drugs. Classical activators bind in the hydrophobic sites of ClpP, while more recently, other activators have been seen to bind in the active sites. Here, we identified synthetic compounds that are able to bind in both sites by utilizing protease degradation assays and X-ray crystallography. We also solved the first structure of a fungal ClpP, both bound and unbound to Dioctatin, a small molecule activator produced in Streptomyces. Dioctatin binds both hydrophobic and active sites of ClpP. Inspired by this phenomenon, we defined the allosteric pathway for ClpP activation by using hydrogen deuterium exchange mass spectrometry (HDX-MS) and molecular dynamics (MD) simulations. Taken together, this work advances our understanding of ClpP allostery, which can aid in drug design and development efforts in the future.
Differential gene expression in the upper respiratory tract following acute COVID-19 infection in ambulatory patients that develop Long COVID

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Background: Post-acute sequelae of COVID-19, or long COVID, is a condition characterized by persistent COVID-19 symptoms. As long COVID is defined by clinical criteria after an elapsed period, an opportunity for early intervention may aid in future prophylactic approaches, however at present, the pathobiological mechanisms are multifactorial. By analyzing early virally infected upper respiratory tract tissue prior to eventual clinical diagnosis, it may be possible to identify biomarkers of altered immune response to facilitate future studies and interventions.

Methods: This is a sub-group analysis of samples collected from those with confirmed COVID-19. RNA extraction from nasopharyngeal/mid-turbinate samples, sequencing, and bioinformatic analysis was performed to analyze long COVID and non-long COVID cohorts at day 14 post infection. Differences in mean viral load at various timepoints were analyzed as well as serological data.

Results: We identified 26 upregulated genes in patients experiencing long COVID. Dysregulated pathways including complement and fibrinolysis pathways, and IL-7 upregulation. Additionally, genes involved in neurotransmission were dysregulated, and the long COVID group had significantly higher viral load, and slower viral clearance.

Conclusion: Uncovering early gene pathway abnormalities associated with eventual long COVID diagnosis may aid in early identification. We show that, post-acute infection, in situ pathogenic deviations in viral response are associated with patients destined to meet consensus long COVID diagnosis that is entirely dependent on clinical factors. These results identify an important biological temporal window in the natural history of COVID-19 infection and long COVID pathogenesis amenable to testing from standard of care upper respiratory tract specimens.
Differential Epitope-Level Type 1 and Type 2 Cellular Immune Responses Elicited by COVID-19 Vaccination in Patients with Chronic Kidney Disease

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Background and Purpose: Chronic Kidney Disease (CKD) patients are immunocompromised and respond poorly to vaccination. As CKD mortality was high early in the COVID-19 pandemic, we performed humoral and cellular immune assays to better understand CKD immune responses pre- or post COVID-19 vaccination.

Methods: The BOOST-KIDNEY clinical trial enrolled 273 CKD participants with peripheral blood sampling at various pre- or post vaccination timepoints. Serology results of this analysis used the clinically-validated Roche Elecsys assay. Peripheral blood mononuclear cells for a subset of participants were cultured with SARS-CoV-2 spike (S1, S2 regions) and nucleocapsid (N) peptides and cytokines measured using the Legendplex flow cytometry assay.

Results and Discussion: CKD participants exhibited increased concentrations of anti-S antibody post-3rd vaccination (p<0.0001). Levels further increased post-bivalent booster (p=0.0078). Hybrid immunity, defined as both infection and vaccine-induced immunity, differed when assessed by anti-N antibodies vs. cellular immunity. The number of participants deemed to have hybrid immunity increased 3-fold (317%) when incorporating anti-N cytokine production in comparison to anti-N antibody alone. Presence of anti-N IFN-γ was associated with a greater IFN-γ response to spike peptides (p=0.0016). Differential epitope level responses were observed by vaccine type where Wuhan strain boosters increased anti-S1 IFN-γ (p=0.0403) and IL-10 (p=0.0015), but not TNF-α and IL-2. In contrast, bivalent boosters increased anti-S2 IFN-γ (p=0.0323), TNF-α (p=0.0015), and IL-2 (p=0.0359) but not IL-10.

Conclusions: Anti-N cellular immunity had greater sensitivity for detecting prior infection than anti-N antibodies which may wane over time. This finding has implications for clinical trial analyses where “never exposed” cohorts as assessed by humoral testing may be misleading. The differential response to different spike epitopes following vaccination with SARS-CoV-2 variant strains supports ongoing evolution of heterogeneous cellular immune responses in CKD patients which should extend to broader based protection against future exposure.
A non-human primate model of human coronavirus HCoV-229E recapitulates aspects of respiratory disease observed in humans

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Background: Seasonal human coronaviruses (HCoVs) account for 10-30% of respiratory tract infections. However, there are relatively few animal models that exist to study pathogenesis and interventions. Non-human primates have been shown to be good models of moderate disease for SARS-CoV-2 suggesting they may also be suitable models for seasonal coronavirus infections. In our study, we describe a non-human primate disease model of infection with the seasonal coronavirus HCoV-229E.

Methods:
Male Rhesus macaques were challenged intranasally with the dose of 1.78 x 10^6 TCID50 of the HCoV-229E virus. Infectious virus was detected throughout the course of infection. In total six animals were included in the study; three were vaccinated with a fowl adenovirus based recombinant SARS-CoV-2 vaccine before challenge in order to evaluate cross-protection.

Results:
Animals developed characteristic signs of an upper respiratory tract infection, including elevated body temperature, mild nasal discharge and tracheobronchial lymph node enlargement. Hematological and biochemical markers showed a decrease in white blood cell counts following infection. Four weeks after infection, the primates were euthanized, and histopathological analysis of the lungs was performed to evaluate the extent of pulmonary disease. Mild fibrosis and inflammation were observed. Interestingly, a group of animals that received the adenoviral-based SARS-CoV-2 vaccine and subsequent challenge with HCoV-229E showed reduced viral shedding and reduced lung pathology, suggesting low-level cross-protection.

Conclusion: Our study demonstrated that the non-human primate model of HCoV-229E infection mimics aspects of disease observed in humans. Additionally, low levels of cross-protection were conferred to animals that received the recombinant fowl-adenoviral SARS-CoV-2 vaccine. This animal model therefore has the potential to expedite the development of vaccines and therapeutics for HCoV-229E as a representative model for seasonal coronaviruses.
Characterization of Human Parainfluenza Virus 3 (HPIV3) infection in ifnar-/- mice as a model for lower respiratory tract infection

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Background: Human parainfluenza viruses (HPIVs) cause significant upper and lower respiratory illnesses in vulnerable populations. HPIV3, the most prevalent among HPIVs, contributes to approximately 18,000 hospitalizations of infants and children in the United States. Despite exposure in childhood, long-lasting protective immunity is not developed, and re-infection can occur. Thus, vaccines and therapeutics are needed. This study aims to establish ifnar-/- mice as a small animal model for parainfluenza infection to study virus pathogenesis and evaluate future vaccine efficacy.

Methods: Five-week-old male ifnar-/- mice were intranasally infected with HPIV3 strain VR-1782, with a dosage of up to 2.0 x 10^6 TCID50. Daily monitoring post-infection involved recording weight and body temperatures. Euthanasia was performed at 1, 4 and 7-days post-infection (dpi) to assess disease severity and progression. Oral swabs, lungs, and nasal turbinates were harvested for RT-PCR and to evaluate infectious viral load. Histopathological analysis was also performed on the lungs.

Results: Mice showed elevated body temperatures at 2-3 dpi compared to uninfected control mice. The viral genome copy number reached its zenith at approximately 4 dpi in the lungs but was low or absent in the oral swabs at all time points, except 1dpi. Histopathological analysis revealed inflammatory infiltrates in lungs of infected mice as early as 1dpi, distinguishing them from uninfected controls.

Conclusions: Our data indicates that ifnar-/- mice mimic aspects of HPIV3 infection observed in humans. Peak viral infectivity is observed around 3-4 dpi, with a subsequent decline in viral load by 7 dpi. Notably, the virus predominantly manifests as a lower respiratory tract infection in these mice. These observations substantiate the establishment of a murine model for HPIV3, providing a valuable platform for future investigations into vaccine development.
Is blood group A-antigen a receptor for SARS-CoV-2 infection?

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Introduction: Blood group antigens have been widely implicated in SARS-CoV-2 infection as a predictor of disease severity. Early pandemic studies observed a protective trend against disease severity in patients that were group O, and a higher risk for group A patients. While the underlying mechanism is not well understood, one theory suggests that the A-antigen is a secondary receptor for the Spike protein of SARS-CoV-2. The Spike protein contains a receptor binding domain (RBD), which is responsible for recognizing and binding to the host cell receptor, ACE2. The RBD has similar sequences to galectins, which are an ancient family of lectin proteins that bind to carbohydrate antigens, including ABH antigens. Supporting this theory, Wu et al. found the A-antigen acts as a receptor for RBD-expressing lentivirus and identified similar sequences within RBD to galectins. Whether A-expression leads to increased infection by live virus was not tested.

Methods: To further explore this, we cotransfected SARS-CoV-2 Spike-expressing plasmid with ABH glycosyltransferases in HEK293T/17 cells, then infected the transfected cells with Spike-pseudotyped lentivirus that also expresses luciferase reporter and measured infection using luciferase read-out.

Results/Conclusions: We observed no difference in infection between A-, B-, or H-antigen-expressing cells, with or without the over-expression of ACE2 in HEK293T/17 cells. Thus, our results do not support the idea that A-antigen is a receptor for SARS-CoV-2 infection. Indeed, our previous work showing that the protective effect seen in group O is likely due to the presence of natural blood group antibodies, since group O individuals produce high titer of IgM and IgG anti-A and anti-B antibodies, while groups A and B only produce IgM anti-B and IgM anti-A, respectively. In summary, we cannot confirm the work of Wu et al. and additional testing using live SARS-CoV-2 viruses is necessary to further investigate this theory.
Realist Evaluation of the Policy Formulation stage in adopting Antimicrobial Stewardship Programs in Indonesia and Pakistan

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Antimicrobial Resistance (AMR) is a global threat. It is estimated that more than 10 million people could die every year, due to AMR, by 2050. Low- and middle-income countries (LMICs) have the highest antimicrobial consumption and AMR prevalence. One key approach to preserving the efficacy of available antimicrobials is implementing Antimicrobial Stewardship (AMS) programs. AMS programs are a collaborative multidisciplinary team operation that requires technical overlapping of scopes of practice among physicians, nurses, and pharmacists. AMS programs adoption in low- and middle-income countries is facing considerable challenges in terms of human resource incapacity, finances, training, turf protection by various professions, lack of context-specific data, influence of the pharmaceutical industry, weak regulatory infrastructure, weak surveillance infrastructure, and many more. This realist study intends to identify and explain the causes of delay in adopting AMS programs. Therefore, the study evaluated a policy formulation stage that negotiated the formulation and adoption of Antimicrobial Prescribing guidelines in Indonesia from 2015 to 2021 and Pakistan, where deliberations have been ongoing since 2017. This study explains the influences that exert pressure on the policy advisory system during the policy formulation phase in two different contexts. It exposes a wider policy environment and provides insight into the various dimensions of professional dominance that affect adopting AMS programs in both countries. The results of this study are original and offer an unprecedented glimpse into the usually obscure policy formulating architecture.
When macrophages bite off more than they can swallow - dealing with Aspergillus fumigatus

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Macrophages are tissue-resident immune cells that play fundamental roles in housekeeping and immunity. Key to these functions is the cellular process of phagocytosis, where macrophages recognize, internalize, and degrade apoptotic cells and invading microorganisms in intracellular compartments known as phagosomes. With time, phagosomes mature by fusion with endo-lysosomes, gaining the capacity to destroy their cargo. In our bodies, macrophages confront targets that not only differ in their surface chemistry but in their size and morphology as well. Indeed, some invading microbes greatly surpass the macrophage's internalizing capacity, and yet, macrophages have been established to play a crucial role in controlling them through unknown handling mechanisms. This is the case with the filamentous fungus Aspergillus fumigatus, an opportunistic respiratory fungal pathogen that, once inhaled into the respiratory tract, can quickly germinate into long invasive hyphae. This transformation is life-threatening unless it is controlled by the respiratory mucosal defenses orchestrated by resident alveolar macrophages. Our work investigates how phagocytosis and macrophage cellular physiology adapt to the containment of non-phagocytosable A. fumigatus hyphae. We found that macrophages form long-lasting engagements with hyphal tips. We characterized this engagement as an open phagosome, gaining markers of maturation, such as reactive oxygen species (ROS) and late endosomal markers Lamp-1 and Rab7. Although we found that open phagosomes fail to acidify, they succeed in stalling hyphal growth, a process dependent on ROS production and cell wall damage. Altogether, our results show that the anti-microbial role of phagocytosis is not limited to targets that can be completely internalized.
Neisseria gonorrhoeae adaptation during vaginal colonization of CEACAM-humanized mice

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Neisseria gonorrhoeae, the causative agent of gonorrhea, is a human-adapted bacterial pathogen. Due to increased incidence of antibiotic resistant gonococcal infections, understanding the molecular mechanisms of infection is vital in search for therapies and vaccines. N. gonorrhoeae has a highly plastic genome, were genomic changes that lead to altered expression and production of virulence factors can occur during infection, as well as through in vitro laboratory passaging. Phase variation, where changes in repetitive sequences can turn genes on/off, exemplifies the capacity of N. gonorrhoeae to adapt rapidly. Understanding N. gonorrhoeae adaptation in a natural niche could identify new targets to combat disease. We aimed to identify genes important in gonococcal vaginal colonization by comparing in vitro passaged ‘lab-adapted’ with in vivo passaged ‘host-adapted’ N. gonorrhoeae. We utilized three distinct clinical isolates in a CEACAM-humanized murine model of gonococcal vaginal colonization. This model allows better engagement of gonococci with epithelial cells and the immune system through gonococcal Opa adhesins and the humanized CEACAM cell-surface receptors. We passaged isolates in vitro to create lab-adapted strains prior to infecting mice in the lower genital tract. Mice were vaginally lavaged to monitor colonization and collect host-adapted N. gonorrhoeae for subsequent re-passaging and whole genome sequencing. After 3 in vivo passages, comparative genomics was performed to identify adaptive mutations. In vivo passaged N. gonorrhoeae showed increased duration of colonization compared to the in vitro passaged strains. The mutation most commonly found in host-adapted strains leads to phase variation of the methyltransferase modA, where it is turned on in host-adapted strains, suggesting its function is important during infection. We hypothesize modA is regulating genes that contribute to the success of N. gonorrhoeae during vaginal colonization. Future work plans to determine the specific mechanisms modA and other genes we identify have on gonococcal fitness and infectivity.
Identifying and characterizing Candida albicans genes that modulate susceptibility to Manogepix

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Invasive fungal diseases are responsible for over 2.5 million deaths globally each year. One of the most common fungal pathogens is Candida albicans, which causes invasive infections with high mortality rates of up to 55%. The arsenal of effective antifungal agents is limited to three major classes, including polyenes, azoles, and echinocandins, with three distinct modes of action, targeting either the plasma membrane or fungal cell wall integrity. Limited treatment options and an alarming rise in drug-resistant isolates urge the development of novel therapeutics. Gepix drugs are novel antifungal agents in development that inhibit fungal glycosylphosphatidylinositol (GPI) acyltransferase Gwt1, essential for GPI-anchor biosynthesis. Manogepix is a first-in-class inhibitor of fungal Gwt1 with broad-spectrum antifungal activity against the major fungal pathogens, including drug-resistant species. Recently, a prodrug of MGX, fosmanogepix, entered phase 3 clinical trials for invasive mycosis treatment, providing hope that replenishing our limited repertoire of antifungal agents is within reach. This work aims to identify and characterize C. albicans genes for which transcriptional repression confers hypersensitivity to MGX. To determine the genes of interest, a high-throughput functional genomic screen was performed, leveraging a large-scale Gene Replacement and Conditional Expression (GRACE) collection of C. albicans mutants with controllable gene expression through a doxycycline-repressible promoter system. Specifically, 3,555 mutants with strain-specific molecular barcodes were grown in a pooled format in the presence and absence of MGX, and strain abundance was measured using high-throughput sequencing. By applying robust computational analyses, we identified 16 genes that modulate susceptibility to MGX, primarily involved in cell wall-related processes, including cell wall macromolecule metabolic process and cell wall organization or biogenesis. Overall, investigating the genetic circuitry underlying fungal susceptibility to MGX will reveal novel mechanisms governing GPI-anchor biosynthesis and other biological processes important for MGX antimicrobial activity.
Identification and characterization of compounds that inhibit filamentation of the human fungal pathogen Candida albicans

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Over the past several decades, there has been a surge in fungal infections, resulting in over 6.5 million individuals suffering from an immediate life-threatening fungal disease annually, directly leading to the deaths of 2.5 million people. Candida albicans, both a commensal of the healthy human microbiota and a leading opportunistic fungal pathogen, represents the most prevalent etiological cause of candidiasis, with a mortality rate of ~40% despite therapeutic intervention. A pivotal virulence trait governing the switch from the commensal to the pathogenic state is the ability of C. albicans to transition between yeast and filamentous forms, as the vast majority of mutants locked in either state are avirulent in mouse models of candidiasis. Due to the close evolutionary relationship between C. albicans and the human host, the number of fungal-specific targets available for antifungal development remains limited, and antifungals that are currently in clinical use are plagued with problems of host toxicity or drug resistance. In light of this, a novel strategy to treat fungal infections is to target virulence traits implicated in pathogenicity without affecting growth—thereby expanding the therapeutic target space while mitigating disruption to the host microbiota and reducing the selective pressure on the pathogen to develop resistance. This work explores this alternative approach by identifying and characterizing compounds that inhibit filamentation of C. albicans. Specifically, we report results from a high-throughput and quantitative, dual-strain screen strategy to distinguish molecules that inhibit filamentation from those that inhibit growth in tissue culture medium (RPMI) at 37 °C. For this strategy, a nourseothricin (NTC)-resistance marker was placed downstream of the filament-specific promoter HWP1pr or downstream of the constitutive promoter TEF1pr, enabling identification of compounds that specifically inhibit filamentation using optical density as a readout. Screening ~50,000 compounds identified three molecules, NPD231, T1, and NPD4271, that were prioritized based on potency, minimal mammalian cytotoxicity, and availability of chemogenomic profiles to help predict compound mechanism. Thus far, we confirmed that all compounds reduce filamentation in response to multiple inducing cues. Furthermore, all compounds blocked filamentation of a strain in which the core filamentation regulator, protein kinase A, was hyperactivated. These results, coupled with available chemogenomic datasets, suggest NPD4271 may function as a cationic amphiphilic compound, disrupting membrane homeostasis, while NPD231 and T1 either act downstream of PKA or through modulation of other cellular processes that underlie filamentation. Future work will be centered around elucidating the mechanisms of action of these compounds, as well as investigating their therapeutic potential as an anti-virulence therapy.
Evaluating Carbapenamase Detection: A Comparative Study of Two Lateral Flow Assays and CIM Across Gram-Negative Bacteria

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Objectives: This study aims to evaluate two lateral flow assays (LFAs), NG-Test® CARBA-5 and Genobio K.N.I.V.O. Detection K-Set, and the carbapenem inactivation method (CIM) for the detection of carbapenamases using well-characterized clinical and reference isolates of various Gram-negative species.

Method: In total, 152 isolates were included this study: 10 Acinetobacter baumannii, 12 Pseudomonas aeruginosa, and 130 from multiple species within Enterobacterales. Among these, 132 harbored a carbapenamase (37 KPC, 36 NDM, 25 OXA-48, 13 VIM, 9 SME, 6 IMP, 2 NMC, 2 INC, and 2 NDM+OXA-48-like) and 20 were negative for carbapenemases. Both LFAs are designed to detect KPC, NDM, VIM, IMP and OXA-48-like carbapenemases from a bacterial colony within 15 minutes. Additionally, LFAs were qualitatively scored for background color and test line indicator intensity. For CIM testing, a 10ul-loop was used to generate the inoculum. A meropenem disk was then immersed in the suspension of the isolate, placed in a Miller Hilton agar plate inoculated with a 0.5 McFarland suspension of E. coli ATCC25922, and incubated for 2 or 4 hours.

Results: For Enterobacterales, NG-Test® CARBA 5 had an overall concordance of 100% while Genobio KNIVO had a concordance of 99.2% because of a false negative IMP-8 producing K. pneumoniae isolate. Moreover, both LFAs demonstrated a concordance of 100% for A. baumannii and P. aeruginosa isolates separately. In terms of the test line intensity, both LFAs were comparable while for background signal, Genobio KNIVO gave more results with a cloudy weak background. CIM provided results with less concordance (2hr/4hr incubation): 40%/40% for A. baumannii, 83.3%/66.7% for P. aeruginosa, and 80%/81.5% for Enterobacterales.

Conclusion: The two LFAs deliver excellent performance in detecting select carbapenemases, offering quick results, straightforward interpretation, and minimal hands-on time. Conversely, the CIM test is labor-intensive, slow, and difficult to interpret. Furthermore, our findings reveal that the LFAs are superior in terms of concordance when compared to CIM which gives sub-optimal performance.

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Background: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Comparison of COVID-19 and MS brain autopsies showed similar neuropathology including astrocytosis, axonal damage, blood-brain barrier leakage and microglial nodules. Preliminary work in our lab revealed the presence of immune cell aggregates in the meningeal layers surrounding the brain of Syrian Hamsters infected with SARS-CoV-2. As such, it is critical to learn whether SARS-CoV-2-induced brain pathology augments MS disease severity or accelerates progression.

Objective: We wish to determine whether SARS-CoV-2 infection augments the neuropathogenic potential of myelin-primed Th17 cells in animal models of MS.

Methods: We have established a working model in humanized ACE2 knock-in (hACE2-KI) mice that combines experimental autoimmune encephalomyelitis (EAE) with SARS-CoV-2 infection. Following resolution of infection with a non-lethal dose of the delta strain of SARS-CoV-2, I induce passive EAE and assess: (1) clinical presentation of both EAE using pre-established scoring systems and (2) CNS pathology.

Results: Mice with prior SARS-CoV-2 infection exhibited reduced incidence of EAE by approximately 50% in experimental groups with prior infections. Additionally, these mice exhibited less severe EAE clinical symptoms during the chronic phase of EAE. Despite this, phenotypic assays reveal the presence of SARS-CoV-2-specific antibody-secreting cells (ASCs) and an accumulation of immune cells within various regions of the brain in these mice. Interestingly, mice with a prior SARS-CoV-2 infection but no EAE exhibited early evidence of inflammation in their brains, but not spinal cords.

Conclusions and Significance: These findings suggest that mild SARS-CoV-2 infection might reduce the impact of encephalogenic Th17 cells on spinal cord damage, but not on the brain. To investigate the impact on the brain, behavioural studies to assess cognition could be performed. With further work in both chronic and remitting animal models of MS and using additional SARS-CoV-2 variants, including omicron, we hope to further understand the impact of SARS-CoV-2 infection on MS disease course and neuroinflammation.
Optimization of small molecules targeting the yeast casein kinase, Yck2, as a therapeutic strategy to combat Candida albicans

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Candida species are a major cause of invasive candidiasis with a mortality rate of ~64% despite treatment. The emergence of antimicrobial resistance coupled with the limited antifungal arsenal highlights the need for novel therapeutics and targeting fungal kinases is a promising avenue. A previous chemical screen revealed a 2,3-aryl-pyrazolopyridine molecule, termed GW, as an inhibitor of C. albicans yeast casein kinase 2 (Yck2). Yck2 is required for growth under physiological conditions, is important for maintaining echinocandin resistance, and plays a key role in virulence in a mouse model of infection. While GW demonstrates potent bioactivity against C. albicans, its poor metabolic stability presents a liability for its progression into in vivo studies. Therefore, we engaged in medicinal chemistry efforts to optimize GW. Two sets of molecules, GW bioisosters containing an imidazo[1,2-a]pyridine scaffold, and structure-guided R-substituents of the parent GW pyrazolo[1,5-a]pyridine scaffold, were generated. Utilizing genetic and biochemical approaches, we characterized dozens of analogs and identified three molecules with improved pharmacological properties, which retained whole-cell bioactivity and selectivity for the fungal Yck2 compared to the human CK1α isoform. Efficacy studies in a mouse model of systemic candidiasis revealed one of our most advanced compounds were capable of potentiating the antifungal activity of a non-curative dose of caspofungin, resulting in significant reductions in kidney fungal burden. Future work will focus on development of Yck2 inhibitors with further improvements in potency, fungal specificity, and pharmacokinetic properties.
Birnaviruses replication: A journey from endosomes to the Golgi


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The infectious bursal disease virus (IBDV) and the infectious pancreatic necrosis virus (IPNV) are major pathogens of food-producing industries. Birnaviruses replicate in the cytosol of host cells, where great amount of dsRNA molecules, foreign to the host cell metabolism, are produced. Host cells have cytosolic sensors of dsRNA that readily initiate immune responses to prevent viral spread. To deal with this challenge, birnaviruses co-opt host endosomes to “hide” and use them as physical platforms for viral replication. Previously, we showed that viral replication occurs in association to the cytosolic leaflet of endosomes, and in close association with the Golgi complex, the later key for viral assembly. Here, we show that IBDV and IPNV replication on endosomes depend on the interaction between the viral protein VP3 and the Phosphatidylinositol-3-Phosphate (PtdIns3P), an early endosomal signaling membrane lipid. We characterized the molecular features of VP3-bearing endosomes and found that these are hybrid organelles that bear molecules of early and late endosomes, but lack degradative capacity. Importantly, we found Rubicon protein, a negative regulator of endosome maturation, on these organelles. If these compartments are not maturing to become degradative organelles; nor being recycled to the plasma membrane, where are these VP3-bearing endosomes going? We also found that VP3-bearing endosomes associate with a subset of molecules from the endosome-to-Golgi retrograde pathway, the sorting nexins 1 and 2. Furthermore, the retrograde trafficking of these endosomes was found to depend on the motor protein dynein and microtubules. We saw that depletion of PtdIns3P or the sorting nexins prevented the endosome to the Golgi journey of VP3-bearing endosomes, and therefore had a great impact on birnaviruses’ infection. In our model, birnaviruses exploit VP3-PtdIns3P interactions to replicate on hybrid endosomes that traffic in a retromeric fashion to ultimately dock at the Golgi complex for viral assembly.
An Analysis Of The Value Of Antiabiogram Subgroup Stratification

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Background: The Infectious Disease Society of America and Clinical and Laboratory Standards Institute recommend the use of stratified antiabiograms to guide empiric clinical treatment. Generating annual unit and specimen-specific susceptibility reports is a laborious task involving large datasets and data manipulation. This stratification is often limited to academic hospital based laboratories due to resource allocation challenges. This study compares differences in antibiotic susceptibility between an accumulative hospital-wide, all-specimens antiabiogram and stratified antiabiograms in order to identify the value added of subgroup stratification of antiabiograms. Method: Antibiotic susceptibility of bacterial isolates from 2021 at a tertiary-care academic hospital was obtained from published accumulative and unit- and specimen-specific stratified antiabiograms. Differences in percent susceptibility by organism and drug between the accumulative and stratified antiabiograms were calculated. A weighted percent susceptibility (WPS) was calculated for the accumulative and each stratified antiabiogram. Heat maps representing differences in WPS were created and differences in WPS were compared for significance using Chi-squared Test. Excel and R Statistical Software were used for graph generation. GraphPad QuickCalcs was used for statistics. Result: Antiabiograms from emergency department (ED) samples had significantly higher susceptibility as measured by WPS whereas intensive care unit (ICU) and transplant (TR) show reduced susceptibility (Figure). Blood isolates and urine isolates were significantly less susceptible compared to hospital-wide isolates. In subgroup analysis, bloods samples showed significantly higher susceptibility in the ED and significantly reduced susceptibility in the ICU. Urine shows higher susceptibility in ED and lower susceptibility in TR. Respiratory samples in TR showed a large reduction in susceptibility. A figure is shown displaying the subgroup antiabiograms to an all-specimen all-units reference antiabiogram (* p ≤ 0.05). Conclusion: There are unit- and specimen-specific differences in susceptibility compared to accumulative hospital-wide antiabiogram susceptibilities suggesting the need for unit- and specimen-specific antiabiograms for optimal empiric antimicrobial management.
Characterizing Lactobacillus iners Diversity in the Vaginal Microbiome

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Dysbiosis of the vaginal microbiome, known as bacterial vaginosis (BV), is associated with negative health outcomes, including HIV acquisition. Unfortunately, BV recurrence after treatment is common. Improved understanding of vaginal microbiome dynamics is needed to better treat and prevent BV. Lactobacillus species, which dominate most vaginal microbiomes, are thought to protect against BV. However, Lactobacillus iners, estimated to be the most prevalent species globally, is thought to be BV-permissive, as it is observed during BV and lacks production of protective metabolites. High intraspecies variation among other Lactobacillus sp., the high prevalence of L. iners, and differing stabilities of L. iners-dominated microbiomes bring forth the possibility that some strains of L. iners stably protect against BV, while others are permissive to BV. Previous difficulties in culturing L. iners has prevented investigation of in vitro phenotype diversity, including growth in co-culture with BV-associated bacteria. I have developed a chemically defined media for improved L. iners growth. Using this media, I isolated bacterial strains from vaginal fluid from sex workers in Nairobi, Kenya. I have developed a pipeline for identifying the species of my isolates and differentiating between strains. I am currently isolating bacteria from participants with L. iners-dominated microbiomes and BV-like microbiomes, and then identifying unique L. iners strains. In the future, I will compare phenotypes of the isolated strains in vitro. I will determine how different host-relevant conditions impact strain growth, and how strains grow in co-culture with each other, other Lactobacillus, and BV-associated bacteria. This work will determine if different L. iners strains are present in different microbiome states, how strains vary, and if strains can outcompete BV-associated bacteria. Together, this will suggest whether all L. iners strains are BV permissive or if some may protect against BV, improving our understanding of what constitutes an optimal vaginal microbiome.
Applied utility of the neisserial Type XI secretion system in Escherichia coli

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The type XI secretion system (TXISS) in Neisseria gonorrhoeae translocates specific substrates across the bacterial outer membrane to be surface-displayed or secreted. Many of these substrates are key virulence factors and putative vaccine antigen candidates, allowing the gonococcus to evade host complement proteins or overcome nutritional immunity defense mechanisms. We adapted the neisserial TXISS to be heterologously expressed in Escherichia coli and engineered these substrates to be secreted as full-length untethered exoproteins to levels that are not possible to scale in the native gonococcal organism. We developed a novel pipeline for expression, purification, and production around this system that have far fewer purification steps and demonstrate significantly better production yields and purities compared to conventional cytoplasmic purification strategies. The secreted substrates have better stability and lower susceptibility to proteolysis compared to their cytoplasmic counterparts and are equally immunogenic in our mice studies.

There are three TXISS translocons in Neisseria gonorrhoeae and we have demonstrated substrate specificity using our system. We produced TXISS secreted substrates to pursue high-resolution structural studies by x-ray crystallography and resolved several new crystal structures in the process. In addition, we have utilized our system to produce substrates that are functionally capable in binding their target proteins in the host, allowing us to capture these large substrate complexes and resolve atomic resolution structures by cryo-electron microscopy. We have used our secretion system to rapidly screen and characterize mutations to better understand the structure-function aspect of these secreted substrates. Finally, we demonstrated that our engineered substrates can be utilized as a secretion export signal in fusion with other proteins, expanding the system’s utility as a cargo-carrying protein production system.
Population genomics analysis of Neisseria gonorrhoeae shows link between population structure, mobile elements & antimicrobial resistance

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The bacterial pathogen Neisseria gonorrhoeae has been designated as a WHO Global Priority pathogen due to the rapid rise of antimicrobial resistant (AMR) strains. A better understanding of underlying population structures and its diverse collection of infection-associated genomic factors will aid in combatting the spread of this rapidly developing public health threat. I have collected a set of over 60 thousand gonococcal genomes deposited in the NCBI Sequence Read Archive suitable for my analysis. These genomes were aligned to a reference strain for identification of SNVs, which were then used to calculate a pairwise degree of difference between each isolate within a geographic region. A maximally diverse set of isolates was collected for each available combination of year and continent of isolation to form a final reference set of 488 genomes. The overall population structure of this diverse set was then determined using PopNet. The genomes of these isolates were then assembled de novo to ensure genes not present in the reference genome were accounted for. I then determined the gonococcal pangenome, from which a core genome and several mobile genetic elements were identified. AMR genes were predicted and compared to existing records of resistance for isolates where applicable. My research shows a strong link between AMR-associated genes and gonococcal clades, indicating the key role AMR plays in shaping the gonococcal population. Future work will include the identification of loci under positive and negative selection pressure to identify key conserved virulence factors, as well as GWAS studies aimed at identifying genes or variants associated with geographic locations, sites of infection and the sex of the host.
Modeling the neuro-inflammation of the spinal cord in a polio-like paralysis, using human immune-competent neural avatars

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Years after the eradication of Poliovirus (PV) from the Western world, recurring outbreaks of Acute Flaccid Myelitis (AFM) have made this disease a significant public health concern. Peaks in cases of AFM have been temporally associated with outbreaks of the non-polio enteroviruses EV-D68. Considering the phenotypic similarities of AFM to poliomyelitis the disease is likely caused by a viral agent. The mechanisms that lead to spinal cord injury and resultant acute paralysis in AFM are unclear. Recent evidence suggests EV-D68 could gain entry to the spinal cord via retrograde axonal transport like PV. However, at the time of AFM diagnosis viral antigens are rarely detected. AFM patients present elevated systemic inflammatory cytokine levels. Inflammatory responses are initiated in response to infection for viral clearance, but excessive cytokine release can lead to neurotoxicity in the absence of direct infection. We hypothesize AFM pathogenesis is a result of direct infection of neural cells, and focal innate cell-mediated inflammatory damage which results from the responses of reactive innate immune cells like microglia and astrocytes. Using human pluripotent stem cell (hPSC) derived cultures, in 2D and 3D, as models of the brain, spinal cord, and peripheral nerves I investigate the cellular and host responses to infection by EV-D68 to delineate the mechanisms of injury in AFM. Our data shows that monocultures of key cell types of the CNS, i.e. neurons, astrocytes, and microglia, are susceptible to infection by EV-D68. We also show that infection of neurons and astrocytes leads to the production of infectious viral progeny. Furthermore, our data illustrates the susceptibility of 3D-multicellular models of the CNS to EV-D68 infection, and using single-cell RNA sequencing (sc-RNA sequencing) we elucidate transcriptomic changes that occur due to inflammasome activation and antiviral defenses.
Investigating the role of CD39 in Mycobacterial tuberculosis infections

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is a significant global epidemic causing 1.3 million deaths in 2022. Currently Bacille Calmette-Guérin (BCG) is the only approved vaccine for TB but has limited efficacy in adults. The cell-mediated immunity programs required for TB protection remain largely unprofiled. We used time-of-flight mass cytometry (CyTOF) on mouse lungs infected with Mtb, BCG, and a vaccine candidate developed in-lab through the loss of the protein Lsr2 (Δlsr2) in Mtb to characterize the cell-type specific immune profiles after various mycobacterial infections. Overall Mtb infected mice displayed the strongest immune response in terms of frequency of innate and adaptive immune cells and expression of cytokines, BCG had the lowest immune response, and Δlsr2 had an intermediate response, reflecting our previous work and the bacterial burden of these mice. After interrogating 38 immune protein markers including cell type, activation, and immune checkpoint markers, we found that CD39 expression in CD4+ and CD8+ T cells was the most strongly associated with bacterial burden of any combination of marker and cell-type. This association persisted across multiple timepoints, different vaccine strategies, and in both male and female mice, highlighting its robust nature. Therefore, we aimed to disentangle whether CD39 impacts Mtb infection and immunogenicity or if CD39 expression is a readout of infection severity. Specifically, we infected mice with Mtb and after three weeks treated with polyoxymetallate-1 (POM-1), a chemical inhibitor of CD39. While we did not see a difference in bacterial burden, we did see an increase in IFNγ producing T cells, a key cytokine for control of Mtb, and an overall increase in the frequency of CD4+ and CD8+ T cells in the lungs of mice treated with POM-1. Future work involves optimizing robust CD39 functional screens and investigating the use of POM-1 as an adjunct therapy with the current antibiotic regimen for TB. Our work predicts a functional relationship between CD39 and Mtb infection, which has strong implications for clinical application through CD39 inhibition in Mtb treatments.
**Development of novel inhalation approaches for robust sterilizing mucosal immunity against SARS-CoV-2 and Variants of Concern**

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Introduction: The Omicron variant has demonstrated the ability to evade neutralization by antibodies generated through vaccination or previous infection, raising concerns about its transmissibility and severity. Notably, mucosal antibody responses, particularly in the airways, may be insufficient following intramuscular mRNA vaccination, potentially contributing to viral escape. Thus, preserving and inducing robust mucosal immunity in the upper airway could be pivotal in combating Omicron. Methods: We assessed the efficacy of decoy strategies, mimicking mucosal receptors and pathogens, in preventing Omicron viral entry. Using human lung organoids engineered in our laboratory, we compared viral replication, tissue tropism, and cytokine induction of SARS-CoV-2. Multiple doses of human recombinant ACE2 (rACE2) and Virus-like particles (VLPs) were administered post-infection. Additionally, a dose escalation study was conducted in humanized-ACE2 mice, with VLPs administered intranasally before SARS-CoV-2 instillation and aerosolized rACE2 given post-infection. Results: Both rACE2 and VLPs exhibited dose-dependent inhibition of SARS-CoV-2 infectivity, reducing viral levels to near undetectable (*P<0.05). Treatment with rACE2 and VLPs ameliorated cytopathic effects in lung organoids, mitigating cell-swelling, detachment, and shedding. In humanized-ACE2 mice, VLP treatment significantly reduced viral load (P=0.029) and titers (P=0.015), accompanied by reduced weight loss (*P<0.05). Daily inhalation of rACE2 for five days protected mice from SARS-CoV-2 infection, decreasing lung viral load. Notably, rACE2-treated mice exhibited improved infection symptoms. Conclusions: Our findings demonstrate the susceptibility of human iPSC-derived lung organoids to Omicron infection and the efficacy of rACE2 and VLPs in preventing viral replication and COVID-19 in humanized-ACE2 mice. These results underscore the potential of rACE2 and VLPs as promising decoy strategies to neutralize SARS-CoV-2 and inhibit viral entry, providing valuable insights for combating Omicron and future variants. Grant acknowledgement: This work was supported by the Canadian Institutes of Health Research (OV3-170334, SBC-171482 and VS1-175560).
Systematic functional screening reveals metaeffector regulation of a multifunctional Chlamydia trachomatis effector

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Chlamydia trachomatis, the causative agent of chlamydia, infects 129 million people/year globally, leading to pelvic inflammatory disease, infertility, and ectopic pregnancy if untreated. It also causes trachoma, the leading cause of infectious blindness around the world.

As an intracellular pathogen, C. trachomatis manipulates host cells through use of over 100 translocated proteins, termed "effectors", facilitating its replication within vacuoles known as inclusions. To balance host manipulation with host survival, some pathogens, including Chlamydia, have evolved "metaeffectors" or "effectors of effectors". These metaeffectors regulate the activity of other effectors through direct interaction. I systematically screened for these interactions by leveraging the phenotypes associated with effector expression in yeast. Among a library of 246 Chlamydia effectors, I identified 10 novel functionally antagonistic interactions, three involving physical interaction, indicative of metaeffector activity.

One particularly intriguing interaction involves the multifunctional effector CpoS and its uncharacterized metaeffector CT214. CpoS is known to suppress host cell death and the type I interferon response, disrupt vesicle trafficking, and interact with other Chlamydia effectors. These functions are facilitated by two distinct coiled-coil domains in CpoS, one binding RAB GTPases and the other binding other C. trachomatis effectors. Here I present a model where, unlike other CpoS-interacting effectors, CT214 can regulate the disruption of vesicle trafficking by CpoS through physical interaction with its RAB-binding domain. I am currently investigating whether this regulatory mechanism extends to other key functions of CpoS. To accomplish this, I am determining how CT214 activity influences CpoS-related phenotypes during infection. I am also developing new methods for proximity labeling during infection to observe how CT214 alters CpoS interactions with host factors and other effectors.

Overall, my work sheds light on a new level of regulation in key pathogenic processes and contributes to the development of novel methodologies that can benefit the broader pathogen research community.
Combating HIV and Influenza through targeting of cellular RNA processing pathways

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From the seasonal influenza to the ever-enduring crisis of HIV, the viruses’ ability to rapidly adapt and resist current therapeutics has become increasingly evident. The existing arsenal of antiviral medications, often targeted to viral proteins such as polymerase or protease in HIV, or neuraminidase for influenza to prevent new viral particles release, have struggled to keep up with the rapid evolutionary adaptation of viruses. Both HIV and Influenza genomes are compact, coding for multiple proteins whose expression relies on alternative RNA splicing for their replication. This requirement for a cellular process presents an opportunity to approach antiviral strategies from a host perspective by targeting RNA processing steps. We hypothesize that modulation of key splicing machineries and their regulators can hinder virus replication. SR (Serine-Arginine) proteins and Heterogeneous nuclear ribonucleoproteins (hnRNPs) are conserved host proteins involved in both host and viral RNA processing. We curated a chemical library of SR kinase inhibitors test our hypothesis. Screening of this library identified several compounds that are able to inhibit both HIV and influenza replication. Here, we present various evidence in the context of HIV and influenza on the capacity of select compounds to reduce viral RNA and protein, and alter alternative splicing site usage that correlate with changes in SR protein phosphorylation patterns. These studies set the stage for the future exploration of inhibitors that target the RNA processing steps as antivirals, potentially leading to a therapeutic breakthrough in the ongoing battle against viral infections.
Launching the novel ‘torpedo’ sampling method for avian influenza viruses in wetlands

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Avian influenza viruses (AIVs) can significantly impact animal health, and they are of continuing concern for future pandemic emergence. The ongoing highly pathogenic avian influenza (HPAI) H5N1 virus outbreak necessitates increased AIV surveillance efforts in Canada. Current methods for surveillance of AIVs rely on animal sampling, which is limited by resources and accessibility. We have developed and tested a novel AIV environmental sampling device, “the torpedo”, that contains sorbent materials allowing for water sampling by immersion and towing from a watercraft.

Sampling was conducted at six Ontario wetlands from 17 August to 20 September 2023. Oral and cloacal swab samples from free-ranging waterfowl were collected in parallel with wetland water samples using the torpedo. All samples were tested by qPCR with universal influenza A matrix gene and H5 gene targets. Samples that were matrix gene-positive and H5-negative underwent whole-genome sequencing using the Illumina MiniSeq platform. H5-positives were sent to the National Centre for Foreign Animal Disease for further analysis.

Two hundred swab samples and 72 torpedo sorbent material samples placed in 28 torpedoes were collected. AIV was detected in 32 swab samples and 16 sorbent materials from 5 torpedoes. Mean Ct values for all samples ranged from 27.18 to 35.96 in waterfowl samples and from 36.57 to 38.92 in torpedo samples. H5 was detected in 1 waterfowl sample with a Ct of 31.72 and no torpedo samples. Whole genome viral sequences were obtained from both swab and torpedo samples, and 7 unique subtypes were detected. All sequenced viruses contained multiple internal genes which have reassorted with H5N1 HPAI. The average sequencing depth was 91.55 for torpedo samples and 544.08 for waterfowl samples. We concluded that the torpedo environmental sampling method is capable of detecting AIV in water in a field setting.
On the role of recombination in viral spillover and emergence

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Many pandemics (SARS-CoV-1 and -2, 2009 H1N1) are thought to have been enabled by pathogen recombination, i.e., the exchange of genetic material during infection of the same host with multiple genotypes. This means that processes which influence the incidence of disease in the host population affect the rate at which pathogen genetic material is shuffled. We develop eco-evolutionary models to investigate how ecological traits of a host (e.g., mean lifetime, immune investment) influence the rate at which pathogen genetic material is recombined, and the consequences of this recombination on the pathogen’s emergence in a novel host. We find support for the idea that pathogens of short lived, acutely infected hosts (e.g., rodents) should recombine frequently. However, even when recombination is extensive, we find that the risk of emergence is greatest when hosts lie on the other end of the life history continuum (e.g., bats). The reason for this is two-fold. (1) Emergence-favoring mutations are found at highest frequency when hosts are long-lived and chronically infected. (2) Although recombination alters associations between mutations, its effects on the risk of emergence is small relative to the other forces at play.
A Parasite Odyssey: An RNA virus concealed in Toxoplasma gondii

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We are entering a “Platinum Age of Virus Discovery”, an era marked by exponential growth in the discovery of virus biodiversity, and driven by advances in metagenomics and computational analysis. Viruses can infect all organisms constituting the microbiome, including bacteria, fungi, and unicellular parasites. Thus the complexity of possible interactions between host, microbe, and viruses is unfathomable. To understand this interaction network we must employ computationally-assisted virology as a means of analyzing and interpreting the millions of available samples to make inferences about the ways in which viruses may intersect human health.

From a computational viral screen of human neuronal datasets, we identified a novel narnavirus Apocryptovirus odysseus (Ao) which likely infects the neurotropic parasite Toxoplasma gondii. Previously, several parasitic protozoan viruses (PPVs) have been mechanistically established as triggers of host innate responses, and here we present in silico evidence that Ao is a plausible pro-inflammatory factor in human and mouse cells infected by T. gondii. T. gondii infects billions of people worldwide and PPVs like Ao could function as a hitherto undescribed hypervirulence factor. In a broader screen of over 7.6 million samples, we explored phylogenetically-proximal viruses to Ao and discovered 19 Apocryptovirus species, all found in libraries annotated as vertebrate transcriptome or metatranscriptomes. While samples containing this genus of narnaviruses are derived from sheep, goat, bat, rabbit, chicken, and pigeon samples, the presence of virus is strongly predictive of parasitic Apicomplexa nucleic acid co-occurrence, supporting that Apocryptovirus is a genus of parasite-infecting viruses.

This is a computational proof-of-concept study in which we rapidly analyze millions of datasets from which we distilled a mechanistically, ecologically, and phylogenetically refined hypothesis. We predict this highly diverged Ao RNA virus is biologically a T. gondii infection, and that Ao, and other viruses like it, will modulate this disease which afflicts billions worldwide.
Realist Evaluation of the Policy Formulation phase in adopting Antimicrobial Stewardship Programs in Indonesia and Pakistan

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IHPME

Antimicrobial Resistance (AMR) is a global threat. It is estimated that more than 10 million people could die every year, due to AMR, by 2050. Low- and middle-income countries (LMICs) have the highest antimicrobial consumption and AMR prevalence. One key approach to preserving the efficacy of available antimicrobials is implementing Antimicrobial Stewardship (AMS) programs. AMS programs are a collaborative multidisciplinary team operation that requires technical overlapping of scopes of practice among physicians, nurses, and pharmacists. AMS programs adoption in low- and middle-income countries is facing considerable challenges in terms of human resource incapacity, finances, training, turf protection by various professions, lack of context-specific data, influence of the pharmaceutical industry, weak regulatory infrastructure, weak surveillance infrastructure, and many more. This realist study intends to identify and explain the causes of delay in adopting AMS programs. Therefore, the study evaluated a policy formulation stage that negotiated the formulation and adoption of Antimicrobial Prescribing guidelines in Indonesia from 2015 to 2021 and Pakistan, where deliberations have been ongoing since 2017. This study explains the influences that exert pressure on the policy advisory system during the policy formulation phase in two different contexts. It exposes a wider policy environment and provides insight into the various dimensions of professional dominance that affect the adoption of AMS programs in both countries. The results of this study are original and provide an unprecedented glimpse into the usually obscure policy formulating architecture.
Very little is known about the impact of intramuscular vaccination on mucosal immune responses. Specifically, whether a mucosal antibody response is induced by COVID-19 vaccination. We recently reported that COVID-19 vaccination elicited a transient mucosal slgA response in healthy adults that rapidly wanes in most, but not all vaccinees. Moreover, vaccinees who had breakthrough infections post-vaccination had lower systemic (serum) IgA antibodies to SARS-CoV-2 spike/RBD. Here, we aimed to characterize the salivary antibody response to vaccination in a cohort of healthy children, and how this response compares to adults. We recruited n=100 healthy 5-11-year-old children and acquired saliva samples at baseline, and various time points post-dose 1 and post-dose-2. We observed significant increases in salivary SARS-CoV-2-specific IgG and IgA levels postdose-1 compared to baseline. Pediatric postdose-1 SARS-CoV-2-specific salivary IgG and IgA levels were 3x and 2x higher than adults, respectively. Salivary IgG levels were further boosted post-dose-2 and, similar to adults, IgA levels dropped significantly compared to post-dose-1, with only 46% of pediatric subjects remaining positive. We believe that the oral microbiome may be one factor influencing the IgA response, and the capacity of certain subjects to retain this response post-dose 2. We have collected preliminary data which shows that anti-RBD IgA levels correlate with levels of IgA-coated bacteria in saliva. We also see that subjects who experienced a delayed breakthrough infection (4-6 months post-dose 2) had higher levels of IgA and IgG-coated bacteria in their saliva at baseline compared to subjects who experienced an early breakthrough infection (1-4 months post-dose 2). We are currently conducting preliminary animal experiments to help us better understand the relationship between the anti-commensal response and the post-vaccine mucosal IgA response in the upper respiratory tract.
Leveraging chemical libraries to identify and characterize molecules with antifungal activity against Candida albicans

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Candida albicans is a leading cause of deadly systemic infections in a growing population of immunocompromised patients, with mortality rates of up to 90% when untreated. The leading class of antifungals used to treat C. albicans infections are the azoles, which block ergosterol biosynthesis by inhibiting lanosterol demethylase encoded by ERG11. Alarmingly, the efficacy of the azoles is hindered by both basal tolerance towards the drug and the development of resistance. Leveraging chemical libraries offers a powerful approach to uncover molecules with novel antifungal activity. Thus, in a quest to identify novel antifungals, we screened two diverse compound collections against C. albicans. The first was a collection of 54,000 compounds from the Broad Institute Diversity-Oriented Synthesis library. Through this endeavour, we discovered a compound termed BRD6758 that through chemical-genetic approaches was predicted to inhibit the ergosterol biosynthetic enzyme Erg11, despite lacking a canonical azole ring. Sterol profiling confirmed treatment of C. albicans with BRD6758 led to an increase in lanosterol levels and a decrease in ergosterol levels, providing additional evidence that the compound inhibits Erg11 activity. The second screen was with the Medicines for Malaria Venture’s library of 640 compounds in various stages of drug development. Through this approach I identified 28 molecules that strongly inhibited growth of C. albicans, six of which have an uncharacterized mechanism of action against fungi. To prioritize hits, I evaluated compound cidality and spectrum of activity against azole-resistant and echinocandin-resistant Candida strains, as well as against other diverse fungal pathogens. The mechanism(s) of action of prioritized compounds are currently being investigated using chemical-genetic approaches. Overall, this project has the potential to reveal and characterize compounds with novel antifungal activities against C. albicans.
Regulation of gut commensal protozoa by micronutrients.

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The intestinal microbiota is one of the richest ecosystems composed of viruses, fungi, bacteria, and protozoa which inhabit many living organisms, including humans. Despite much emphasis on bacteria, the role and behavior of gut protozoa remain largely undefined. While pathogenic protozoa have been reported, a neglected commensal protozoa termed Tritrichomonas musculis (T.mu) was recently found in the gut of mice, with close human orthology. Our investigations identified that T.mu exists across three life cycle stages, covering a growing trophozoite stage, an adherent ameboid stage and a pseudocyst stage. Our dietary intake heavily influences the function and development of intestinal microbes. For example, iron as dietary micronutrient guides host physiology via metabolism, growth, and immunity, and impacts the bacterial microbiota. Whether iron impacts the metabolism, growth, or life cycle stages of T.mu remains an open question. This project investigates the role of iron supplementation and depletion on the T.mu pseudocyst stage. We hypothesize that modulation of environmental iron impacts pseudocyst formation of T.mu in vitro and in vivo. We utilize culturing of freshly isolated T.mu in anaerobic chambers, followed by cell counting, and flow cytometry of new pseudocyst markers. In addition, we establish a proof-of-concept system to determine whether T.mu pseudocysts can colonize the host. Ultimately, we aim to determine the importance of environmental factors on T.mu biology and further our understanding of their dynamic life cycle stages on the intestinal ecosystem.
Defining the molecular and developmental impact of preimplantation viral infection

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Upwards of 8% of the mouse and human genomes are comprised of genetic material derived from viruses. These endogenous retroviruses (ERVs) are intrinsically expressed during the earliest stages of preimplantation embryonic development. ERVs contribute alternate promoters, encode protein products, and modulate chromatin accessibility in ways that are required for normal embryonic development. After peaking at the 2-cell embryo (2C) stage, ERV expression is strictly repressed through the rest of development and for the rest of the normal life course except in rare circumstances, or in cases of disease.

Given the unique regulation of ERVs during embryonic development, we asked how embryonic cells would respond to exogenous retroviral infection. We used the Murine Stem Cell Virus (MSCV) to infect mouse embryonic stem cells (mESCs). Although mESCs normally do not express most ERVs, we found that MSCV infection induced expression of 2C-specific ERVs. ATAC-sequencing also showed that the 2C-specific ERV family MERVL had more accessible chromatin across thousands of genomic insertions. We then used a MERVL reporter cell line to show that retroviral producer plasmid DNA was also able to activate 2C-like transcription, yet viral RNA extracted from virus or synthesized through in-vitro transcription could not. Our work suggests that exogenous retroviral infection may activate 2C-like transcriptional programs. Whether this is an adaptive or deleterious response remains an open question, and our next steps will be to pinpoint transcriptional activators and repressors that may be involved in this process. This work will advance our understanding of how preimplantation embryos respond to viral infection.
Elucidating mechanisms of resistance and susceptibility in wild isolates of C. elegans against distinct microsporidia species

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Microsporidia are rapidly emerging opportunistic infectious pathogens that infect humans and most other organisms including agriculturally important species such as honeybees and fish. Several parasitic microsporidian species have been isolated in wild strains of C. elegans. Using PhenoMIP, a multiplexed sequencing-based screen for measuring phenotypes in C. elegans, 22 wild strains were tested against four microsporidian species. We identified two strains, JU1400 and MY1, which are sensitive to an epidermal-infecting species, yet resistant to an intestinal-infecting species. Complementation tests between JU1400 and MY1 suggests these strains share variants in the same genes that are responsible for sensitivity and resistance. Using genetic mapping, we identified four distinct loci which may be responsible for these differential phenotypes. Generation of introgressed lines narrowed critical region of the sensitivity phenotype to 700kb on the left arm of Chromosome I4. I plan to test genes with shared variants in MY1 and JU1400 to identify the causative genes responsible for their susceptibility phenotype. Concurrently, I plan on performing a forward genetic screen to identify genes responsible for the resistance phenotypes. Finally, I will expand the PhenoMIP assay to examine a greater number of wild isolates to identify additional strains with variations in fitness. With a larger panel of wild strains, causative variants can be identified through genome-wide-association studies. Overall, we will identify key molecular mechanisms in host-pathogen interactions between C. elegans and microsporidia that serves as a model to understand these types of infections in humans.
Anti-phage defence through inhibition of virion assembly


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Bacteria and their viruses (bacteriophages) are locked in a perpetual arms race that has given rise to an impressive defensive and offensive arsenal. Temperate phages integrate their genome into the host chromosome as a latent prophage and exhibit a symbiotic relationship with their host by expressing genes that increase bacterial fitness. Since phage predation is a major threat to bacterial survival, prophages have evolved proteins that protect host cells from further phage infections. We showed that phages infecting Pseudomonas aeruginosa frequently express genes that protect their host from phage attack. Since prophages are common, understanding these defence systems and how they protect their bacterial host will provide important insight into the phage-host evolutionary arms race. Here, we identify a type of antiphage defence that interferes with the virion assembly pathway of invading phages. The protein that mediates this defence, which we call Tab (for ‘Tail assembly blocker’), is constitutively expressed from a Pseudomonas aeruginosa prophage. Tab allows the invading phage replication cycle to proceed, but blocks assembly of the phage tail, thus preventing formation of infectious virions. While the infected cell dies through the activity of the replicating phage lysis proteins, there is no release of infectious phage progeny, and the bacterial community is thereby protected from a phage epidemic. Prophages expressing Tab are not inhibited during their own lytic cycle because they express a counter-defence protein that interferes with Tab function. Thus, our work reveals an antiphage defence that operates by blocking virion assembly, thereby both preventing formation of phage progeny and allowing destruction of the infected cell due to expression of phage lysis genes. Since prophages are common in bacteria, and they contain a large untapped reservoir of hypothetical genes, novel antiphage defences are likely widespread. Studies of these systems will reveal important knowledge about the microbial world.
Trends in antimicrobial resistance among Gram-negative organisms in Toronto, Canada between January 2013 and December 2023 – A notable rise in MDRO and XDRO Klebsiella pneumoniae

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The emergence of multi-drug resistant gram-negative organisms (MDR-GN) is a growing threat. There is a need to understand the current trends to inform empiric treatment. This study analyzes the trends in MDR-GN between January 2013 and December 2023 in a tertiary-care academic clinical laboratory serving four acute care hospitals in Toronto, Canada.

Susceptibility testing results were collected for all Enterobacterales (avg. n = 6733/yr), Pseudomonas aeruginosa (avg. = 1312/yr), and Acinetobacter spp. (avg. n = 55/yr). Among Enterobacterales, predominant species included Escherichia coli (avg. n = 4619/yr) and Klebsiella pneumoniae (avg. n = 1221/yr). For each category of organisms, an individual patient was represented only once per year.

Adhering to CPHLN and CACMID guidelines, Enterobacterales spp. isolates were classified as either multi-drug resistant (MDRO), or extensively-drug resistant (XDRO), while P. aeruginosa and Acinetobacter spp. isolates were classified as XDRO. 95% confidence intervals were calculated using the modified Wald method with test for trends calculated using Chi-square.

Over the past decade, there was a 2.5-fold increase in multidrug resistance in K. pneumoniae hospital-wide (5.2% to 13.2%, p<0.001). This trend was primarily driven by MDRO organisms, which also increased by 2.5-fold (3.7% to 9.5%, p<0.001). Although non-significant, there may be an emerging trend of increasing XDRO hospital-wide, as well as of increasing total resistance in the ER and ICU. This is particularly concerning, based on recent reports of the emergence of resistant hypervirulent K. pneumoniae in EU/EAA countries in Feb 2024. Whole genomic sequencing to better characterize both sequence types and resistance profiles is underway.

Total resistance among Enterobacterales and E. coli increased significantly hospital-wide. Similar to K. pneumoniae, this trend was also driven predominantly by MDRO organisms. Additionally, rates of resistance for both Enterobacterales and E. coli were significantly greater in the ICU than in the ER across for most years. This reflects higher rates of multi-drug resistance in acutely ill individuals with higher antimicrobial exposures compared to community-spread infections.
Molecular chaperones and proteases exist in all organisms where they play a critical role in maintaining cellular protein homeostasis. ClpP is one such protease present in both bacteria and eukaryotes. It is composed of fourteen identical subunits that typically assemble as stacked heptameric rings to form a hollow barrel-like structure with 7-fold symmetry. Chemical interference may be used to activate ClpP and dysregulate its function, resulting in the unregulated proteolysis of non-substrate proteins, causing cell death. As such, targeting ClpP has recently emerged as a promising avenue for the development of novel antimicrobial drugs. Classical activators bind in the hydrophobic sites of ClpP, while more recently, other activators have been seen to bind in the active sites. Here, we identified synthetic compounds that are able to bind in both sites by utilizing protease degradation assays and X-ray crystallography. We also solved the first structure of a fungal ClpP, both bound and unbound to Dioctatin, a small molecule activator produced in Streptomyces. Dioctatin binds both hydrophobic and active sites of ClpP. Inspired by this phenomenon, we defined the allosteric pathway for ClpP activation by using hydrogen deuterium exchange mass spectrometry (HDX-MS) and molecular dynamics (MD) simulations. Taken together, this work advances our understanding of ClpP allostery, which can aid in drug design and development efforts in the future.
Toward Comprehensive Annotation of Host-Virus Life Cycle Pathways in Reactome: A Molecular Perspective on Infectious Disease

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Reactome is a manually curated and peer-reviewed knowledgebase of biological pathways, used globally for biomedical and bioinformatics experts to better understand the molecular underpinnings of their research. Recently, Reactome was expanded to annotate host-virus life cycle pathways, which intricately delineate the molecular events underlying viral infection, representing discrete processes within the human body's molecular landscape. Reactome's infectious disease annotation encompasses a diverse array of viruses, including SARS-CoV-1, SARS-CoV-2, HIV, Influenza, HCMV, and RSV.

The annotation of SARS-CoV-1 infection, including viral lifecycle, host interactions, and therapeutic pathways, was achieved through a rigorous curation process. This involved categorizing viral proteins and their interactions with host factors, delineating the intricate signaling cascades and cellular processes hijacked by the virus to propagate within the host. Multinational collaborative efforts with the C19 Disease Map Community facilitated robust pathway refinement of SARS-CoV-2 pathways, capitalizing on shared structural and functional features between the two viruses. This involved integrating data from experimental studies, clinical trials, and computational analyses to prioritize and annotate pathways with therapeutic relevance.

Reactome's serves as an invaluable resource for infectious disease research, fostering interdisciplinary collaborations across diverse viral pathogens. Reactome pathways provide an indispensable framework for dissecting emerging therapeutic strategies and elucidating the impact of viral mutations on infection dynamics. Reactome's broad platform of curated human molecular pathways is an ideal platform for analyzing the interaction of viral mediated pathology and human response. Importantly, Reactome's pathways are a collaborative effort with the broader scientific community, ranging from Peter Palese's and Adolfo Garcia-Sastre's Labs at Mount Sinai for influenza, to a consortium of HIV researchers during Cold Spring Harbor Laboratory Conferences, to a multinational effort to curate SARS-CoV-2. Looking forward, collaborations with the research community remain paramount as Reactome continues to serve as a cornerstone resource for deciphering viral-mediated pathology and human immune responses.
Investigating protein-protein interactions during TIFA-dependent pro-inflammatory signaling

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Innate immune detection of pathogens relies on our cells’ ability to recognize conserved microbial molecules through the use of pattern recognition receptors, and trigger inflammation. ALPK1-TIFA signaling, a recently-discovered innate immune pathway, detects bacterially-derived heptose phosphates (HPs), which are a conserved class of metabolic intermediates produced by Gram-negative bacteria. ALPK1 recognizes cytosolic HPs as a signal of replicating intracellular bacterial pathogens, which triggers TIFA phosphorylation and oligomerization into a ‘TIFAsome’, ultimately activating NF-κB-driven inflammation. While the ALPK1-TIFA-NF-κB signaling axis has been defined, little is known about how the pathway is regulated, and what antibacterial responses it triggers beyond NF-κB activity. In order to expand our understanding of TIFA signaling and its cellular consequences, we utilized proximity-induced biotinylation (BioID) to identify TIFA interactors recruited following HP treatment. To do this, we tagged TIFA with a biotin ligase (BirA) that generates a 'cloud' of activated biotin, allowing us to covalently label nearby proteins and identify them via mass spectrometry. We observed that HP-driven signaling recruited RNF31, a linear ubiquitin ligase, to TIFA. RNF31 promotes NF-κB signaling, and mediates clearance of intracellular bacteria, such as Salmonella, through antibacterial xenophagy. Given that RNF31 and TIFA both respond to intracellular bacterial infection, we sought to understand the RNF31-TIFA interaction and its impacts on inflammation and bacterial clearance. Using an RNF31 knockout cell line, we were able to establish that RNF31 promotes HP-driven NF-κB activity, and to show that HP treatment drives linear ubiquitination. Further work will interrogate how TIFA-RNF31 interaction mediates inflammation and antibacterial xenophagy using a human colonic epithelial cell lines expressing and knocked out for RNF31 to model infection with Salmonella, as well as other Gram-negative intracellular pathogens that activate TIFA signaling, such as Shigella, and adherent-invasive E. coli.
Investigating the regulation of anti-phage systems in Pseudomonas aeruginosa

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The growing threat of bacterial infections has caused pressure to develop novel therapeutics. There is increased interest in using bacteriophages (phages) as an alternative to antibiotics. Phages are viruses that specifically infect, replicate within, and kill bacteria through lysis. Unlike antibiotics, they can precisely target different pathogens with less microbiome disruption. To harness and engineer phages effectively as therapeutics, we need to understand how bacteria resist infection. One barrier to phage therapy is bacterial anti-phage defences. Most bacteria encode several defence systems, providing multi-layered resistance. Some defences are upregulated at high cell density by quorum sensing (QS), a cell-to-cell communication system that regulates genes when cells are in a community, most vulnerable to phage outbreaks. This is thought to be evolutionarily advantageous as production of anti-phage defences is energetically costly, and cells are at risk of self-targeting if systems are expressed in the absence of phage infection.

Pseudomonas aeruginosa is a pathogen and model organism for QS. P. aeruginosa strain PA14 contains four predicted anti-phage defence systems: Shango, Shedu, Wadjet, and Gabija, but no evidence of their activity has been observed. We compared the ability of phages to infect P. aeruginosa PA14 at low vs. high bacterial density and identified phages that could no longer infect. We plated phages on defence systems knockouts and observed infection by certain phages in the absence of Shango and Gabija. Furthermore, these phages could infect QS knockout strains. We identified that QS drives expression of Shango and Gabija in P. aeruginosa, and future investigation will determine whether the network drives the expression of other systems. This has revealed important information about regulation of anti-phage systems in P. aeruginosa and can uncover more on bacterial defence regulation. In the long term, this will contribute to developing effective phage therapeutics that evade defences.
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