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2025

Microbiology and Infectious Diseases Research Days

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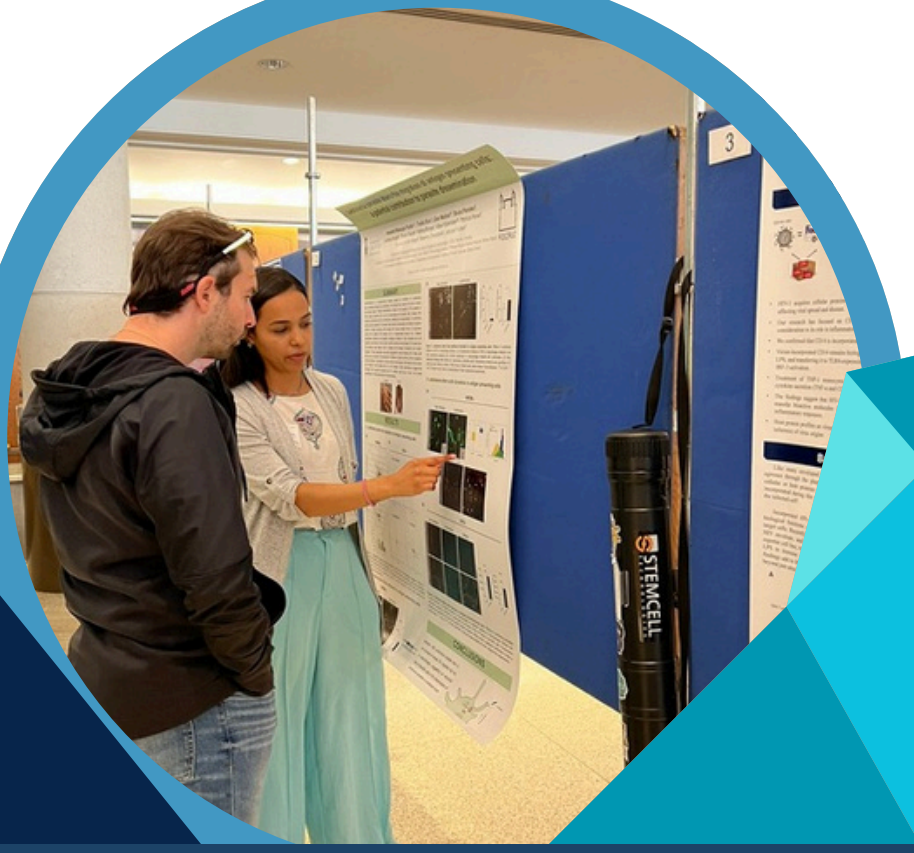
Abstract Booklet



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 AND FLASH TALKS

May 26, 2025
Trainee Day

Medical Sciences Building
Room 3154

The adamantane diamine SQ109 exerts antifungal activity through inhibition of squalene synthase in the fungal pathogen Candida albicans

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Candida albicans is a leading cause of deadly systemic fungal infections in a growing population of immunocompromised patients. Azoles are the most common antifungal class used to treat these infections, acting by blocking ergosterol biosynthesis through inhibition of the lanosterol demethylase encoded by ERG11. Alarmingly, the efficacy of the azoles is hindered by the frequent development of resistance. Leveraging repurposed chemical libraries offers a powerful approach to uncover molecules with antifungal activity, expediting drug development and lowering regulatory barriers and cost. Thus, in a quest to identify novel antifungals, we screened the Medicines for Malaria Venture's library of 640 compounds in various stages of drug development. Through this approach we identified SQ109 (MMV687273), an antitubercular drug, as a molecule with broad-spectrum activity against C. albicans, Nakaseomyces glabratus, Cryptococcus neoformans, Aspergillus fumigatus and the dermatophyte Trychophyton indotineae. Mechanistic studies indicated that SQ109 inhibits the C. albicans squalene synthase, encoded by ERG9. Specifically, genetic repression of ERG9 caused hypersensitivity to SQ109. In addition, inhibition of Erg9 led to an increase in lipid droplet accumulation, indicative of altered lipid homeostasis, and sterol profiling by GC-MS confirmed SQ109 treatment results in a decrease in downstream sterol intermediates, including squalene, lanosterol, and ergosterol. While robust Erg9 inhibition blocked C. albicans growth, sublethal concentrations of SQ109 impaired C. albicans virulence traits including filamentous growth and biofilm formation. Furthermore, in co-culture assays with mouse monocyte-macrophage lineage J774A.1 cells, treatment with sub-growth inhibitory concentrations of SQ109 rescued macrophage viability through the inhibition of intraphagosomal C. albicans filamentation. Work is now in progress to explore the therapeutic efficacy of SQ109 in a mouse model of systemic infection with C. albicans. Overall, this work characterizes SQ109 as a promising drug for repurposing as an antifungal due to its previously uncharacterized activity against diverse pathogenic fungi.

See also Poster 13

From data providers to data leaders: developing community-led quantitative research capacity using HIV/STI program data in Kenya

Nancy B Tahmo (1), Anthony Noah (2,3), Byron Odhiambo (2,4), Charles Kyalo (2,4), Elly Ondiek (2,5), Fortune Ligare (2,6), Gilbert Asuri (2,3), Jedidah Wanjiku (2,7), John Alex Njenga (2,8), John Maina (2,7), Kennedy Mwendwa (2,6), Kennedy Olango (2,9), Kennedy Ouma (2,9), Loice Nekesa (2,7), Pascal Macharia (2,7), Silvano Tabbu (2,5), Kristy CY Yiu (10), Robert Lorway (11), Parinita Bhattacharjee (12,13), Huiting Ma (10), Lisa Lazarus (11), Sharmistha Mishra (10,14,15), Jeffrey Walimbwa (2,4,16*), on behalf of the Health Research Intervention Kuthamini Afya Yetu Community-Led Research Initiative^

1 Division of Epidemiology, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; 2 Health Research Intervention Kuthamini Afya Yetu (HEKA), Kenya; 3 An Empowered Just and Inclusive Society (AMKENI) Malindi, Kilifi, Kenya; 4 IshtarSHTAR, Nairobi, Kenya; 5 Kenya Youth Development and Education Support Association (KYDESA), Nakuru, Kenya; 6 HIV & AIDS People's Alliance (HAPA) Kenya, Mombasa, Kenya; 7 Health Options for Young Men on HIV/AIDS & STI (HOYMAS), Nairobi, Kenya; 8 Q-Initiative, Eldoret, Kenya; 9 Men Against AIDS Youth Group (MAAYGO), Kisumu, Kenya; 10 MAP Centre for Urban Health Solutions, St. Michael's Hospital, Toronto, Ontario, Canada; 11 Institute of Global Public Health, Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; 12 Institute of Global Public Health, University of Manitoba, Winnipeg, Manitoba, Canada; 13 Partners for Health and Development in Africa, Nairobi, Kenya; 14 Institute of Health Policy, Management and Evaluation, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; 15 Department of Medicine and Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada; 16 Community Research and Technical Support Hub, Nairobi, Kenya

Background: Despite progress in participatory HIV/STI research overall, meaningful community leadership in quantitative research remains limited. Gaps are especially evident in studies with data routinely collected from HIV/STI health program data. The HEKA initiative was established by seven community-based organizations implementing HIV/STI prevention programs among gay, bisexual, and other men who have sex with men in Kenya. HEKA aimed to shift community roles from mere data providers to active leaders and users of quantitative data.
Methodology: HEKA's guiding framework combined principles from the Greater and Meaningful Involvement of People Living with HIV/AIDS and Community-based Program Science. Twenty-one community members, primarily program managers and monitoring and evaluation staff, collaboratively engaged in three iterative capacity-building in-person workshops focusing on quantitative research skills, with virtual engagement between workshops. Academic partners supported community teams through structured mentorship, co-developed skills development sessions, and training in R programming. Together, we cleaned and harmonized the routine program data to set the foundation to address community-defined questions and support program delivery.
Results: Through sustained capacity-building activities, community researchers developed essential quantitative research competencies. These included data management, analysis, visualization, and scientific communication. Community partners successfully led the cleaning of 6 years of program data and preliminary analyses that generated actionable insights to guide HEKA's research strategy. A critical reflection highlighted misalignment between funder-imposed program indicators and local priorities, advocating for the creation of community-defined metrics. Community partners also developed stronger confidence and leadership in identifying research questions and using quantitative evidence.
Conclusions/Significance: HEKA demonstrates the feasibility and substantial value of authentic community leadership in quantitative HIV/STI research. Empowering communities with quantitative research skills has the potential to enrich program relevance and for communities to independently seek research funding. This transformative model offers a framework for shifting research paradigms, underscoring that communities can—and should—lead quantitative research processes.

Systematic functional screening reveals metaeffector regulation of a multifunctional *Chlamydia trachomatis* effector

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1) Department of Molecular Genetics, Temerty Faculty of Medicine, University of Toronto
2) Department of Biochemistry, Temerty Faculty of Medicine, University of Toronto
3) Department of Microbiology, Harvard Medical School

Chlamydia trachomatis is the causative agent of the sexually transmitted infection, chlamydia, which affects 129 million people globally each year. As an intracellular pathogen, C. trachomatis modulates hosts through the collective action of over 100 proteins (“effectors”) that are translocated into host cells during infection. These effectors manipulate host processes, establishing a replicative niche within vacuoles known as “inclusions”. However, to prevent excessive harm to the host, some pathogenic bacteria, including Chlamydia, have evolved “metaeffectors”—which regulate other effectors within the host cell through direct interaction, ensuring balance between host manipulation and host survival. Leveraging phenotypes associated with effector expression in yeast, we systematically screened for these interactions in a pairwise genetic screen of 246 C. trachomatis effectors. Through this, we identified 10 novel functionally antagonistic interactions, three of which were metaeffector interactions.

One particularly intriguing interaction targets the multifunctional effector CpoS (Chlamydia promoter of Survival), an effector that targets host Rab-GTPase proteins during infection. Loss of CpoS activity results in destabilization of the inclusion leading to premature host cell death, reduced pathogen replication in cell culture, and increased clearance from the genital tract. Here, we present a regulatory model where the uncharacterized effector, CT214, physically displaces host Rab-GTPases from CpoS at late stages of infection leading to CpoS inhibition. Consistent with our model, we observed that overexpression of CT214 during infection results in a significant reduction in inclusion size. Considering these observations, we hypothesize that CT214 expression and function during late infection disrupts CpoS-mediated inclusion stability to allow for host cell escape and re-infection of surrounding cells. To test this hypothesis, we are now leveraging recent advances in Chlamydia genetics to understand the role CT214 plays during late infection by generating ct214-null strains and characterizing the consequences of metaeffector loss on host protein recruitment, inclusion stability and pathogen replication.

EPIC Future Leader

Remodeling the Front Line: Salmonella Exploits Host Cell Membrane Dynamics for Invasion

Hongxian (James) Zhu

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Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

Salmonella is a major global pathogen responsible for a wide range of diseases, from gastroenteritis to systemic infections. A key to its virulence is the ability to manipulate host cellular machinery to invade, survive, and replicate within epithelial cells. My doctoral research focuses on uncovering the molecular interface between Salmonella and host plasma membrane (PM) dynamics during the earliest stages of infection. This work began with the identification of RAB10, a small GTPase, as a critical regulator of PM remodeling during bacterial entry. Salmonella was found to exploit RAB10-positive membrane reservoirs (pre-existing tubular PM-associated structures) to orchestrate a coordinated sequence of events including ruffle formation, exocyst complex assembly, and membrane mobilization, ultimately bacterial engulfment and invasion. Further investigation revealed that LRRK2, a kinase implicated in Parkinson's disease and inflammatory bowel disease, modulates these reservoirs through RAB10 phosphorylation. Strikingly, Salmonella activates a novel TLR4/PIEZO1/TMEM16F signaling pathway to trigger LRRK2 inhibition and RAB10 dephosphorylation, promoting membrane scission and bacterial uptake. My research improves our understanding of how Salmonella causes disease and opens up new opportunities for developing therapies that target the specific mechanisms used by the bacteria to infect host cells. It also suggests potential genetic links between bacterial infections and human diseases. By studying how pathogens manipulate host cells at the molecular level, we can create more effective, targeted treatments to fight infections and prevent further disease development.

EPIC Future Leader

A Significant Contribution of the Classical Pathway of Complement in SARS-CoV-2 Neutralization of Convalescent and Vaccinee Sera

Patrick Budylowski (1), Serena L Chau (2), Arinjay Banerjee (3), Furkan Guvenc (4), Reuben Samson (4,5), Queenie Hu (5), Lindsey Fiddes (6), Laurie Seifried (5), Gary Chao (7), Megan Buchholz (8), Antonio Estacio (9), Patti Lou Cheatley (8), Katerina Pavenski (8,10), Christopher J Patriquin (2,11), Yanling Liu (7), Salma Sheikh-Mohamed (7), Kimberly Crasta (7), FengYun Yue (2), Maria D Pasic (12), Karen Mossman (3), Anne-Claude Gingras (4,5), Jennifer L Gommerman (7), Götz R A Ehrhardt (7), Samira Mubareka (13), Mario Ostrowski (1,2,7,9)

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Although high titers of neutralizing Abs in human serum are associated with protection from reinfection by SARS-CoV-2, there is considerable heterogeneity in human serum-neutralizing Abs against SARS-CoV-2 during convalescence between individuals. Standard human serum live virus neutralization assays require inactivation of serum/plasma prior to testing. In this study, we report that the SARS-CoV-2 neutralization titers of human convalescent sera were relatively consistent across all disease states except for severe COVID-19, which yielded significantly higher neutralization titers. Furthermore, we show that heat inactivation of human serum significantly lowered neutralization activity in a live virus SARS-CoV-2 neutralization assay. Heat inactivation of human convalescent serum was shown to inactivate complement proteins, and the contribution of complement in SARS-CoV-2 neutralization was often >50% of the neutralizing activity of human sera without heat inactivation and could account for neutralizing activity when standard titers were zero after heat inactivation. This effect was also observed in COVID-19 vaccinees and could be abolished in individuals who were undergoing treatment with therapeutic anti-complement Abs. Complement activity was mainly dependent on the classical pathway with little contributions from mannose-binding lectin and alternative pathways. Our study demonstrates the importance of the complement pathway in significantly increasing viral neutralization activity against SARS-CoV-2 in spike seropositive individuals.

See also Poster 24

Safety and immunogenicity of adjuvanted subunit respiratory syncytial virus (RSV) vaccination in high-risk transplant recipients.

Adrian Alexander, Faranak Mavandadnejad, Madeline Kern-Smith, Rujun Kang, Blandine Dang, Pascal Lavoie, Sapna Humar, Poramed Winichakoon, Rochelle Johnstone, Meghan Aversa, Igor Novitzky Basso, Atul Humar, Deepali Kumar, Jonas Mattsson, Victoria G Hall, Victor H Ferreira.

UHN

Purpose: Lung transplant (LT) and allogeneic hematopoietic cell transplant (alloHCT) recipients are at increased risk of severe lower respiratory tract disease and mortality from RSV infection. Immunocompromised populations were excluded from the published clinical trial of the approved adjuvanted RSV vaccine, RSVPreF3 (Arexvy, GSK). To address this evidence gap, we evaluated the safety and immunogenicity of RSVPreF3 in LT and alloHCT recipients.
Methods: We conducted a non-randomized, open label, interventional study at the University Health Network (NCT #06593210). Stable outpatients ≥3 months post-LT or ≥6 months post-alloHCT received a single dose of RSVPreF3. Blood was collected at baseline and 4 weeks post-vaccination. Anti-prefusion F IgG levels were measured using electrochemiluminescence immunoassay, with seroconversion defined as a ≥4-fold increase in antibody level. RSV-specific T-cell responses were assessed via flow cytometry following overnight peptide stimulation of peripheral blood mononuclear cells (PBMCs) and identification of antigen-specific polyfunctional T-cells. Safety was evaluated through participant symptom diaries and standardized interviews on days 7 and 42.
Results: A total of 86 participants (40 LT, 46 alloSCT) were enrolled. The vaccine was well tolerated; no rejection or GVHD events occurred. Anti-prefusion IgG levels increased significantly in both groups (LT: p=0.0087; alloSCT: p=0.0030), with median fold-changes of 1.4 (LT) and 2.1 (alloSCT). Seroconversion was observed in 37% of LT and 29% of alloSCT recipients. In alloSCT, seroconversion was associated with a longer time from transplant (p=0.016). RSV-specific CD4+ T-cell responses increased in both groups (LT: p=0.018; alloSCT: p=0.0002), while CD8+ T-cells increased only in LT (p=0.016). At 4 weeks, 72% (LT) and 81% (alloSCT) had detectable CD4+ T-cells; 62% and 52%, respectively, had detectable CD8+ T-cells.
Conclusion: The RSVPreF3 vaccine was safe and elicited robust CD4+ T-cell responses in both lung and alloHCT recipients, although seroconversion rates were modest. Given the importance of antibodies in protection against RSV, strategies to enhance humoral immunity in transplant recipients are warranted. Alternate strategies to enhance humoral immunity, including a second dose of vaccine, warrant further investigation.

EPIC Future Leader see also Poster 67

Profiling miRNA Changes in Epstein-Barr Virus Lytic Infection Identifies a Function for BZLF1 in Upregulating miRNAs from the DLK1-DIO3 Locus

Ashley M. Campbell, Victoria C. Taylor, Beata Cohan and Lori Frappier

Department of Molecular Genetics, University of Toronto, Toronto, Canada

Cellular and viral miRNAs are thought to play important roles in regulating Epstein-Barr virus (EBV) latent and lytic infections, 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, the main sites of lytic infection, we conducted miRNA-sequencing 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. 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 have pro-viral effects, as inhibiting all three together reduced EBV lytic protein expression. Interestingly, these miRNAs all originate from the DLK1-DIO3 locus (14q32.1 - 32.31), which encodes multiple lncRNAs. We showed that the lncRNAs MEG9, MIR381HG, and MEG8, from which miR-409-3p, miR-381-3p and miR-370-3p are derived, were also upregulated upon reactivation in AGS and nasopharyngeal carcinoma cells lines and occurred very early in the lytic cycle at the time of BZLF1 expression. In keeping with this timing, BZLF1 was sufficient to induce these lncRNAs dependent on its transactivation activity, and was detected at a key DLK1-DIO3 control element, consistent with a direct role in transcriptional activation. Therefore, we have identified a new role for BZFL1 in activating the expression of lncRNAs in the DLK1-DIO3 locus, resulting in induction of a subset of encoded miRNAs that promote lytic infection.

Visiting Scientist

Clinical characteristics and exposure patterns among individuals evaluated for Mpox disease in Nigeria.

Evaezi Okpokoro, Edward Kombu, Ekele Peter Ekele, James Onyemata, Darrell H. S. Tan, Sharmistha Mishra, Asukwo Onukak, Biobelu Abaye, Olawole Ayorinde, Isaac Oladefuwa, Daodu Oluwafemi, Sylvia Adebajo, Alash'le Abimiku, Rosemary Audu

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Mpox is endemic in Nigeria with about 984 cases confirmed between 2022-2024. Despite this, case detection is delayed by limited diagnostic. Thus, clinical screening and exposure history are required. Here, we explored the clinical characteristics and exposure patterns among individuals evaluated for Mpox disease in Nigeria.
Method - We conducted a cross-sectional study (06/2023–12/2024). Individuals presenting with fever and rash provided skin lesion specimens and blood for monkeypox virus PCR. Also, individuals previously diagnosed with mpox within 3months were also enrolled. Among these clinical history and exposure to mpox were obtained. We performed descriptive analysis using median and proportions and compared confirm cases to suspects. Also, Chi-square test for association was performed with a p-value of 0.05.
Results - Of the 237 participants enrolled, 19 were diagnosed with mpox and 218 were suspected cases. The median age was higher among cases (24years) compared to suspects (15years) though not statistically significant (p= 0.29). There were fewer males among confirmed cases compared to suspected cases (50% vs 60%, p=0.07). Among 9 individuals diagnosed with acute Mpox, fever (22.2% vs 46.8%; p=0.19), and skin rash (55.6% vs 76.6%; p=0.22) were less common symptoms reported at clinic presentation. However, muscular pains were numerically more common among individuals diagnosed with Mpox (33.3% vs 20.6%; p=0.45). As regards exposure to Mpox, self-reported close contact (26.3% vs 51.9%) or sexual contact (10.5% vs 4.7%) with someone with a similar rash did not differ between cases compared to suspected mpox. The only significant difference between the groups was that cases were more likely to report consumption of bush meat (31.6% vs 10.1%, p=0.014).
Conclusion - The inclusion of muscular pain to clinical screening of mpox may improve case detection. Our findings suggest risks of zoonotic transmission. However, further research is needed to understand myalgia and animal spillover exposure risks in Nigeria.

Mechanism of APOBEC3B Relocalization by Epstein-Barr virus BORF2

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APOBEC3 proteins are a family of cytosine deaminases involved in viral restriction through mutation of replicating genomes. We have previously shown that BORF2, the large ribonucleotide reductase subunit for Epstein-Barr virus (EBV), binds APOBEC3B (A3B) and relocalizes it out of the nucleus into cytoplasmic bodies, protecting lytically replicating EBV from A3B-induced mutations. Similarly, BORF2 homologues from KSHV (ORF61) and HSV-1 (UL39) were found to relocalize A3B into cytoplasmic bodies. However, the mechanisms by which this relocalization occurs remain unclear. It was reported that BORF2 homologues in murine cytomegalovirus and HSV-1 use a motif (the induced protein aggregation motif or IPAM) to induce aggregation of cell signalling proteins. Since the IPAM is conserved in BORF2 and ORF61, we investigated its importance for A3B relocalization. Mutation of this motif was found to abrogate the ability of BORF2, ORF61, and UL39 to bind and relocalize A3B. Staining with ProteoStat showed that, while UL39-A3B bodies were aggresomes, BORF2-A3B and ORF61-A3B bodies were not. Therefore the IPAM is key to A3B interactions and relocalization by different BORF2 homologues, although the A3B body compositions differ. Additionally, we identified a SUMO-modified site in BORF24, which is of interest because the formation of some cellular bodies is dependent on SUMOylation. Although mutation of this SUMO site did not disrupt BORF2 binding to A3B, it did disrupt the formation of BORF2-A3B bodies in lytic infection, suggesting the importance of BORF2 SUMOylation for A3B relocalization. In addition, during EBV lytic infection, a proportion of nuclear BORF2-A3B bodies (before relocalization into the cytoplasm) colocalized with SUMO, reinforcing the potential importance of SUMOylation. Therefore, we have identified residues contributing to A3B relocalization by BORF2 and its homologues, providing insight into the mechanisms by which herpesviruses neutralize A3B to protect their genomes.

Assessing the Health System Impact of an H5NX Influenza Pandemic in Ontario, Canada: A Microsimulation Model

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Institute of Health Policy, Management and Evaluation, University of Toronto

Background: Avian influenza viruses of the H5NX subtype pose a growing threat of zoonotic spillover and potential human pandemic due to increasing mammalian adaptation, highlighting the urgency of preparedness planning. We estimate the impact of an H5NX pandemic on health system resources in Ontario to inform response strategies and resource allocation.
Methods: We developed a discrete event simulation model to simulate trajectories of adult individuals infected with H5NX. Daily infection counts were generated using a compartmental Susceptible–Infected–Recovered model. Transition probabilities were sampled from beta distributions, and lengths of stay (LOS) were modeled using gamma distributions, calibrated to match empirical targets. Health system outcomes, including ward and ICU bed occupancy, mechanical ventilator usage, deaths, and antiviral use, were tracked until simulation completion, with results aggregated over 1,000 simulation replications. LOS results were summarized as median values with corresponding interquartile ranges (IQR). Analyses were conducted in R version 4.4.3 using the simmer package.
Results: The model projected an outbreak of 127,061 cases over the course of a year, resulting in 56,686 deaths (case fatality rate: 44.6%). Demand for acute care resources peaked between days 275 and 350 from the start of the pandemic. The peak resource utilization was 9,975 for ward beds, 12,020 for ICU beds, and 2,831 for mechanical ventilators. Antiviral use was estimated at 118,930 Oseltamivir courses, assuming a full course was provided to each individual presenting to the emergency department. Median hospital LOS was 14.1 days (IQR: 7.7–25.4), with longer stays among survivors—15.6 days (IQR: 8.8–28.1)—compared to non-survivors at 13.0 days (IQR: 7.0–23.6).
Conclusion: The projected burden of an H5NX pandemic would exceed Ontario's acute care capacity, underscoring the need for early resource planning. This analysis demonstrates the value of patient-level microsimulation for evaluating system-wide impacts of emerging infectious disease threats.

Integrative Prediction and Benchmarking of Protein-Protein Interactions in *Plasmodium falciparum* Gametocytes

Khairatun Yusuff (1), Bhavish Verma (1), Koji Wong (1), Grant L. Stevens (1), John Parkinson (1,2)

1) University of Toronto
2) The Hospital for Sick Children

Malaria remains one of the world's deadliest infectious diseases. The WHO's latest World Malaria Report estimates that there were about 263 million cases and 597,000 malaria-related deaths globally in 2023, an increase of 11 million cases from the previous year. Understanding the molecular mechanisms driving parasite transmission is crucial for developing effective interventions. With this goal, we focused on stage-specific protein-protein interactions (PPIs) in Plasmodium falciparum gametocytes, the sexual form of the parasite essential for transmission. Using co-elution mass spectrometry data and protein embeddings from the ESM-2 language model, multimodal deep learning models were developed to predict PPIs in stage 3 and stage 5 gametocytes. True positives were drawn from curated experimental data, and true negatives were generated from co-elution proteins, excluding known interactions. For each stage, five models were trained, validated, and tested using different data splits, and predictions were combined using optimized probability thresholds. The stage 5 model had 2344 predicted interactions amongst 382 proteins, in 84 complexes, and the stage 3 network included 556 interactions amongst 157 proteins in 33 complexes. Network analysis showed that most proteins had a degree of 1, and network hubs included proteins like PfGRP170, an endoplasmic reticulum protein that may contribute to antimalarial drug resistance. Benchmarking using co-expression profiles from RNA-seq data showed significantly higher correlation in expression of predicted PPIs compared to random pairs (Kolmogorov-Smirnov $p < 10\text{-}137$ in stage 5). Ongoing work includes applying phylogenetic profiling to examine co-occurrence patterns across eukaryotic species, detect co-evolved protein pairs, and identify species-specific gains or losses within interaction networks. We also plan to use AlphaFold3 to assess 3D conformations and structural plausibility of predicted interactions. This integrative approach will help prioritize biologically meaningful PPIs and complexes for experimental validation and support the development of stage-specific, transmission-blocking malaria interventions.

Flash Talks

Disruption of Bacterial Biofilms by Genetically Modified Stem Cell-Derived Macrophages: See also Poster 2

Michael Litvack

SLAM - a molecular multipurpose tool for diagnostic innovations: See also Poster 18

Dixon Ng

Therapeutic Efficacy of INT131 in an EcoHIV Mouse Model of HIV-Associated Neurocognitive Impairment: See also Poster 19

Celene Titus

Characterizing pathogenicity differences among *Gardnerella* in the vaginal microbiome: See also Poster 22

Jhenielle Campbell

Investigating the differential vulnerability of microglia and astrocytes to HHV-6A exposure, and the impact of selective infection on neuronal function: See also Poster 31

Daniela Cobo

Multi-omics analysis of the maternal gut microbiome reveals functional changes during pregnancy: See also Poster 34

Grace Parish

Phylodynamic modeling of Seasonal Respiratory Viruses: Influenza A and Respiratory Syncytial Virus circulated in 2023-24, Ontario, Canada: See also Poster 37

Xin Wei1

Ecological correlates of the gut resistome following antibiotic treatment: See also Poster 39

Eric Armstrong

Exploring the metabolism of *Neisseria gonorrhoeae* through genome-scale metabolic modelling

Duncan Carruthers-Lay

POSTER PRESENTATIONS

May 27, 2025
Main Program

Medical Sciences Building
Room 3154

NOTE

Even Numbers are in Session A
Odd Numbers are in Session B

1

A New Mouse Model of HIV in Pregnancy: the EcoHIV Pregnancy Model

Michelle Ranjbar (1), Ingrid Hseih (1), Maud Collomb (2) …, Lena Serghides (1,2)

1) University of Toronto, Canada
2) University Health Network, Canada

Background: Children who are born HIV-exposed but uninfected (HEU) are the fastest growing HIV-affected population, numbering at over 16 million children globally. There is a large gap in our understanding of how in utero exposure to HIV and antiretrovirals influence the development and long-term health of these children. A mouse HIV pregnancy model would greatly facilitate such research.
EcoHIV is an HIV virus that has been modified to express the envelope protein from the murine leukemia virus to allow infection in mouse immune cells. Here we present a new EcoHIV infection model in a mouse pregnancy.

Methods: 7-week-old C57BL/6 mice were infected with 2.5x106 pg EcoHIV virus or mock infected and were mated 1 week post infection. Pregnant dams were euthanized on gestational day (GD) 14.5 or 18.5 (N=5/group). Tissue and blood were collected. HIV infection was detected through HIV gag expression by qPCR. mRNA expression of neuroinflammatory markers were assessed in fetal brains (N=24/group). Mann-Whitney test was used for statistical comparisons.

Results: Injection with EcoHIV resulted in 100% maternal infection, confirmed by HIV gag detection in spleen tissue of all dams. Infection was detected in 4.7% of EcoHIV exposed fetuses. EcoHIV infection was associated with significantly lower fetal weights, lower placenta efficiency, and lower viability rates. mRNA levels of the inflammatory marker TNFa were increased, and mRNA levels of synapsin 1 and synaptophysin, both markers of neuronal health, were decreased in brains of EcoHIV exposed fetuses compared to controls.

Conclusions: The EcoHIV pregnancy model represents the first mouse pregnancy model that includes HIV. In utero EcoHIV exposure was associated with fetal growth restriction and placental insufficiency even in the absence of fetal infection, paralleling clinical findings. Neuroinflammation and neuronal toxicity in EcoHIV exposed uninfected fetuses, may represent a mechanism contributing to neurodevelopmental deficits seen in children who are HEU.

2

Disruption of Bacterial Biofilms by Genetically Modified Stem Cell-Derived Macrophages

Michael Litvack, Sajad Sadat, Deepa Raju, Perrin Baker, Nate Zeeb, Leah Kuo, P Lynne Howell and Martin Post

The Hospital for Sick Children

Alveolar macrophages (AMs) are long living immune cells of the lungs that consume and kill bacteria in the airways. We have used stem cells to create alveolar-like macrophages (ALMs) that, in an animal model, can be delivered directly to diseased airways and repair lung damage and reduce injury from bacterial and viral infection. These cells are like native AMs but can be made in the lab and genetically manipulated to enhance their therapeutic potential. AMs in people with cystic fibrosis (CF) can't effectively consume and kill bacteria in the airways because of thick mucus and matrix encased bacterial clumps called biofilms, which prevent environmental factors such as immune cells and antibiotics from killing the bacteria. Pseudomonas aeruginosa (PA) bacteria that colonize airways of people with CF produce a glycoside hydrolase enzyme called PslG, which can promote the disruption of these biofilms. We genetically modified our ALMs to secrete active PslG. We have found that these PslG-ALMs can disrupt and break mature biofilms and boost the effectiveness of antibiotics. The PslG-ALMs were effective at disrupting biofilms from both lab and clinical isolates of PA – even those isolates noted to be antimicrobial resistant. PslG has a half-life of only 18 hours upon pulmonary delivery; however, we found that direct airway delivery of PslG-ALMs resulted in the detection of active soluble PslG in the airways for several days. Taken together, this study describes a new strategy for targeting antimicrobial resistance that capitalizes on the combined use of stem cell technologies and immunoengineering.

3

The Role of ABO Blood Groups in SARS-CoV-2 Infection

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COVID-19, caused by SARS-CoV-2, requires coronavirus Spike (S)-protein, host receptor ACE2 for infection. Emerging progeny virus use host plasma membrane, which may contain ABH(O) antigens, to form envelopes. Multiple studies reported that blood group O protects against severe COVID-19 disease, while group A patients show increased susceptibility. This suggests that anti-A from group O patients could provide natural protection against COVID-19.

ABH-expressing CoV2-Spike (CoV2-S) lentivirus is produced in HEK293T cells by co-transfecting a luciferase-reporter lentiviral vector, ABO/FUT1 glycosyltransferases, and CoV2-S plasmids. The resulting lentivirus is pre-treated with ABO antibodies, then used to infect target cells (HEK293TACE2+), and infection is measured by luciferase read-out. Both IgM and IgG ABO antibodies will be examined as well as a role for complement activation to elucidate the mechanism of inhibition.

ABH expression on immunoprecipitated S-protein from transfected HEK293T has been confirmed and ABH-specific inhibition with IgM-class monoclonal ABO antibodies has been shown in CoV2-S lentivirus. IgM-class anti-A and anti-B specifically inhibited only A- or B-expressing CoV2-S, respectively. Neither antibody inhibited wildtype and H(O)-antigen expressing CoV2-S. Additionally, pre-COVID-19 plasma from group O, but not group AB, inhibited A- and B-antigen expressing CoV2-S but not H(O)-antigen expressing CoV2-S. IgG-class monoclonal anti-A and anti-B did not inhibit any ABH-expressing or wildtype CoV2-S lentivirus, likely due to lower avidity.

Future work will focus on testing IgG-class polyclonal ABO antibodies in CoV2-S to check if IgG with higher avidity can neutralize the virus. Preliminary work with complement showed it was able to enhance inhibition of IgM anti-A, but not IgM anti-B, which will also be further explored. Additionally, live SARS-CoV-2 will be propagated in ABH-transfected HEK293TACE2+ and tested for ABO-specific inhibition via RT-qPCR and cytopathic effect (CPE). Our study highlights a crucial role of ABO in coronavirus epidemiology, which could aid in managing future outbreaks.

4

Estimates of HPV Vaccination in Canadian Children: Data from the 2021 Childhood National Immunization Coverage Survey

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Introduction/background: Human papillomavirus (HPV) is the most prevalent sexually transmitted infection, causing anogenital warts and several types of cancer. School-based HPV vaccination programs were first introduced by Canadian provinces/territories for females (2007-2011) and subsequently became gender-neutral in all jurisdictions (2013-2017). Despite national targets of 90%, HPV vaccine coverage estimates often fall short. Understanding how vaccination differs by sociodemographic factors is key to increasing coverage.

Methods: We analyzed data from the 2021 Childhood National Immunization Coverage Survey (cNICS). Data were collected via self-response electronic questionnaires or telephone interviews based on a representative sample stratified by child's province/territory of residence and age. Survey sampling weights were applied to ensure the representativeness of the Canadian population of children within the target age range. Parents/guardians of 14-year-olds were asked about their child's HPV vaccination status and sociodemographic characteristics. We calculated the proportion of children vaccinated overall by sociodemographic variables.

Results and analysis: Of a weighted sample of 413,255 respondents, 74.9% indicated their child received the HPV vaccine. Coverage was higher in girls (80.0 %) compared to boys (69.9%), and in Quebec (81.3%) compared to Ontario (74.0%) and all other provinces/territories (72.1%). Urban participants reported higher coverage (75.4%) than rural residents (72.1%), as did respondents born in Canada (77.6%) compared to those born outside of Canada (70.2%). Coverage was also highest among parents with greater education and in the highest household income quintile. Fewer Indigenous children had received the HPV vaccine (55.8%), compared to non-Indigenous children (75.6%).

Conclusions and implications for policy, practice, or additional research:

HPV vaccine coverage varied by sociodemographic factors, with all estimates falling short of the 90% target. Our study demonstrates the importance of examining correlates of HPV vaccination to identify subgroups who may require tailored intervention."

5

Advanced AI-Driven Methane Emission Detection, Quantification, and Localization in Canada: A Hybrid Multi-Source Fusion Framework

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Methane (CH4) is a potent greenhouse gas with near-term climate impact, but accurate detection, quantification, and localization remain major technical challenges. Conventional monitoring, relying on in-situ sensors and single-source satellite data, often suffers from limited coverage, retrieval uncertainties, and poor source attribution. To address these gaps, this paper proposes a novel hybrid multi-source fusion framework that integrates Sentinel-5P satellite data, ERA5 climate reanalysis, and geospatial intelligence from OpenStreetMap (OSM) and Google Earth Engine (GEE). By incorporating atmospheric, meteorological, and industrial features, the framework leverages three tiers of data fusion—feature-level, spatial-temporal, and hybrid modeling—to enrich heterogeneous datasets for high-resolution, AI-driven methane emission monitoring across Canada. It employs deep learning architectures and ensemble-based regressors, including CNN-GRU, LSTM-CNN, and LSTM+XGBoost, to capture complex spatial and temporal dependencies. Experimental results show that hybrid models outperform standalone approaches in anomaly detection and quantification, surpassing 92% classification accuracy and significantly lowering prediction error. Among tested architectures, CNN-GRU achieved the lowest RMSE and highest R^2 for methane concentration predictions. Geostatistical methods like Kriging and IDW, combined with wind-informed KDTree analysis, enabled robust source localization. The resulting scalable and interpretable solution demonstrates enhanced spatial resolution, anomaly classification, and emission attribution. By uniting satellite observations with industrial and environmental data, it offers policymakers and environmental agencies an effective tool to detect, assess, and mitigate methane emissions. This advanced approach fosters synergy between remote sensing and ground-based data, allowing real-time identification of leaks. This integrated methodology paves the way for stronger regulatory frameworks and impactful climate mitigation strategies.

6

The adamantanyl diamine SQ109 exerts antifungal activity through inhibition of squalene synthase in the fungal pathogen *Candida albicans*

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Candida albicans is a leading cause of deadly systemic fungal infections in a growing population of immunocompromised patients. Azoles are the most common antifungal class used to treat these infections, acting by blocking ergosterol biosynthesis through inhibition of the lanosterol demethylase encoded by ERG11. Alarmingly, the efficacy of the azoles is hindered by the frequent development of resistance. Leveraging repurposed chemical libraries offers a powerful approach to uncover molecules with antifungal activity, expediting drug development and lowering regulatory barriers and cost. Thus, in a quest to identify novel antifungals, we screened the Medicines for Malaria Venture's library of 640 compounds in various stages of drug development. Through this approach we identified SQ109 (MMV687273), an antitubercular drug, as a molecule with broad-spectrum activity against C. albicans, Nakaseomyces glabratus, Cryptococcus neoformans, Aspergillus fumigatus and the dermatophyte Trychophyton indotineae. Mechanistic studies indicated that SQ109 inhibits the C. albicans squalene synthase, encoded by ERG9. Specifically, genetic repression of ERG9 caused hypersensitivity to SQ109. In addition, inhibition of Erg9 led to an increase in lipid droplet accumulation, indicative of altered lipid homeostasis, and sterol profiling by GC-MS confirmed SQ109 treatment results in a decrease in downstream sterol intermediates, including squalene, lanosterol, and ergosterol. While robust Erg9 inhibition blocked C. albicans growth, sublethal concentrations of SQ109 impaired C. albicans virulence traits including filamentous growth and biofilm formation. Furthermore, in co-culture assays with mouse monocyte-macrophage lineage J774A.1 cells, treatment with sub-growth inhibitory concentrations of SQ109 rescued macrophage viability through the inhibition of intraphagosomal C. albicans filamentation. Work is now in progress to explore the therapeutic efficacy of SQ109 in a mouse model of systemic infection with C. albicans. Overall, this work characterizes SQ109 as a promising drug for repurposing as an antifungal due to its previously uncharacterized activity against diverse pathogenic fungi.

7

L. crispatus and *L. iners* Differ in Sensitivity to Iron

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Colonization of the vaginal microbiome by Lactobacillus species is thought to protect against bacterial vaginosis (BV) and thus promote host health. In contrast to more diverse microbiomes at other body sites, vaginal microbiomes are typically dominated by a single Lactobacillus species – for most women, either L. crispatus or L. iners. L. crispatus-dominated microbiomes are thought to be more protective against BV than those dominated by L. iners. Why individuals differ in their dominant Lactobacillus, as well as why L. crispatus and L. iners do not tend to co-colonize, is not well understood. Failure of probiotics to colonize subsets of patients suggest that some microbiomes are resistant to colonization by L. crispatus, though whether this is due to host or microbial factors is unknown. Understanding differences between the two species and how to promote the growth of L. crispatus could lead to improved BV treatment and prevention. L. iners does not grow in L. crispatus cultivation media, which has made comparing the species in vitro challenging. I have developed liquid media that supports rapid growth of L. iners and L. crispatus, as well as the BV-associated bacteria Gardnerella. Using this media to compare species' response to various nutrients, I have found that iron inhibits growth of both L. iners and Gardnerella strains at significantly lower concentrations than L. crispatus. I am currently investigating the mechanism underlying this growth inhibition. This will improve our understanding of how the biology of L. iners and L. crispatus differ, and provide one possible explanation for why individuals differ in dominant Lactobacillus. Additionally, this work will suggest if iron could be leveraged to promote L. crispatus growth over that of L. iners and Gardnerella for BV prevention.

8

You win some, you lose some; gene loss as a pathoadaptive strategy in *Shigella flexneri*

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Shigella is a Gram-negative bacterium and the etiologic agent for shigellosis which is a leading cause of diarrheal deaths. The genus Shigella is a polytomous lineage that has emerged multiple times from ancestral strains of Escherichia coli following the acquisition of the ~200-220 kb pINV virulence plasmid. The virulence plasmid mediates the Shigella spp. virulence phenotype through regulation of a type III secretion system (T3SS), encoding genes for host cell invasion and intracellular replication.
Alongside pINV acquisition, conserved gene loss plays a key role in the pathogenesis of Shigella spp. Many of these lost genes are considered “antivirulence genes” as their normal function interferes with the Shigella virulence phenotype. Previous work in our lab has found that multiple Shigella species exhibit conserved loss of multiple two-component signaling systems (TCS) that are typically conserved in E. coli strains. One such signaling system is the citAB/dpiAB system. In E. coli, the citAB/dpiAB genes control citrate utilization under anaerobic conditions. Additionally, it also interferes with plasmid inheritance and gene expression of AT-rich plasmids in E. coli. Based on previous results, we hypothesize that citAB/dpiAB interfere with downstream gene regulation and plasmid inheritance in Shigella.
We have reintroduced citAB/dpiAB into S. flexneri M90T and are assessing its effects on pINV plasmid inheritance, T3SS expression, cellular invasion capacity, and metabolic activity. Our findings indicate that citAB/dpiAB interferes with glycolysis and the Entner-Doudoroff pathway, leading to a pronounced fitness defect under microaerophilic conditions and reduced competitive ability against wild-type strains. Additionally, preliminary data indicates impaired cellular invasion and replication capacity.
Currently, we are assessing impact of the citAB/dpiAB TCS on plasmid inheritance, epithelial cell invasion and overall proteome expression in Shigella. Our work will add to a growing body of evidence highlighting the role of antivirulence genes as significant evolutionary pressures driving genetic heterogeneity among Enterobacterales species.

9

Development of a Human Parainfluenza Virus (HPIV) Matrix Protein-Based Vaccine Elicits Protection Against HPIV Type 3 in Mice

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Human parainfluenza viruses (HPIVs) cause respiratory infections, particularly in vulnerable populations like infants and the immunocompromised. HPIV-3 alone leads to around 18,000 hospitalizations annually in the U.S. The matrix (M) protein of HPIVs is highly conserved, making it a promising target for cross-protective vaccines. This study aimed to establish a mouse model for HPIV-3 infection and evaluate the protective effects of vaccination with HPIV-3 M protein. Two studies were conducted. In the first, C57BL/6 and ifnar-/- mice were intranasally challenged with HPIV-3, strain C243 (2 x 106 TCID50), and monitored for symptoms. Mice were euthanized at 1-, 4-, or 7-days post-infection (dpi), with tissues from the lungs and nasal turbinates collected for viral load assessment, histopathology, and RNA sequencing. In the second study, C57BL/6 mice were vaccinated with 5 µg of HPIV-3 M protein and an adjuvant. Twenty-one days later, they were challenged with HPIV-3. At 4 dpi, nasal turbinates and lungs were evaluated for viral load and histopathology. In the first study, both C57BL/6 and ifnar-/- mice had peak viral loads in the lungs at 4 dpi. Histopathology showed lung inflammation with macrophages and neutrophils, and RNA sequencing revealed upregulation of antiviral and inflammatory genes. In the second study, vaccinated C57BL/6 mice showed no symptoms, lower viral loads, reduced titers, and less severe lung pathology compared to unvaccinated controls. This study establishes a reliable mouse model for HPIV-3 infection, demonstrating similar viral dynamics and immune responses in both C57BL/6 and ifnar-/- mice. Vaccination with the HPIV-3 M protein offers strong protection, reducing lung pathology and viral titers. These results support the M protein as a potential therapeutic vaccine candidate, and further studies are needed to optimize formulations and assess long-term immunity. Ongoing experiments aim to replicate these findings in ifnar-/- mice.

10

Investigating regional differences in respiratory epithelial responses to respiratory syncytial virus infections using human airway models

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INTRODUCTION: The respiratory tract is a complex ecosystem frequently targeted by viral pathogens, with RSV as the primary cause of lower respiratory infections in children. RSV can escalate to severe conditions like acute respiratory distress syndrome (ARDS) if it spreads to the lung's peripheral regions. Despite its public health impact, the clinical progression of RSV infections is not well understood. The lung epithelium, the first line of defense, plays a crucial role in immune response and disease pathogenesis. Its spatial heterogeneity, from proximal to distal regions, influences infection response, affecting viral replication and disease severity. We hypothesize that RSV's impact will vary across nasal, proximal, and distal airway regions.
METHODS: We differentiated wildtype iPSCs into proximal and distal lung epithelial cells and infected them with RSV-GFP using various MOIs to identify the optimal infection level. Immunohistochemistry visualized the infection, while quantitative PCR and flow cytometry measured viral load. Ciliary beating frequency and epithelial barrier integrity were monitored to assess cellular responses.
RESULTS: RSV infection was tracked by increasing GFP signal, with an MOI of 0.5 effectively replicating viral load in both proximal and distal lung regions. Disruption of tight junctions and increased CBF were observed in infected proximal cells. Additionally, successful RSV infection in distal epithelial cells was observed.
CONCLUSIONS: This study optimized RSV infection models for proximal and distal lung regions, identifying an effective MOI for future research. iPSC-derived lung epithelial models confirmed RSV's ability to infect these cells, providing a robust platform for further studies on RSV infections.

11

Exploring coinfection interactions using within-host energy partitioning models

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Parasites rely on hosts for resources during an infection, while hosts require nutrition to mount an immune response against infection. This often puts hosts and parasites in competition for resources. Previous mathematical modeling shows that the structure of resource flows (or energy partitioning) within a host influences the outcome of infections for both hosts and parasites. Put another way, increasing resource intake can benefit either the host or the parasite, depending on the structure. Importantly, hosts in nature are often infected with more than one parasite strain or species, meaning that coinfecting parasites may also compete with each other for resources. Here we extend previous work to understand how resource flows influence the outcome of coinfections. Using generalized mechanistic models, we first identify resource partitioning structures that actually permit coinfection. Second, we determine the extent to which coinfection alters predictions about when increasing resources is good for a host. Third, we examine whether and how resource flows alter the consequences of coinfection compared to single infections for hosts and parasites. Of particular interest are scenarios where coinfection has a disproportionate (negative) impact on one parasite, opening up the possibility that treating the other parasite could lead to worse outcomes for hosts. Understanding within-host interactions between coinfecting parasites and their hosts is essential for predicting the off-target effects of interventions aimed at a single parasite.

12

The Baseline Vaginal Immune Milieu is a Strong Determinant of the Inflammatory Response Induced by Penile-Vaginal Sex

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Penile-vaginal sex immediately induces cervicovaginal epithelial disruption and HIV-associated markers for 48-72 hrs, but there is considerable heterogeneity in the degree of inflammation following sex. We hypothesized that the degree of sex-induced inflammation would be dependent on the pre-existing vaginal immune milieu, with an enhanced inflammatory response in participants exhibiting baseline vaginal immunoquiescence. We employed multiplex immunoassays to quantify HIV-associated soluble immune factors and antibodies within cervicovaginal secretions of HIV-uninfected, STI-free Canadian women before and 1hr after condomless (n=29) or condom-protected (n=8) penile-vaginal sex. Both IgA and IgG significantly increased after sex in cervicovaginal secretions ($p=0.0017$ and $p<0.0001$, respectively), and baseline cervicovaginal IgA was associated with vaginal inflammation ($p=0.0093$) and epithelial disruption ($p=0.0379$). While all soluble immune factors at baseline were positively associated with their absolute concentrations after sex, post-sex concentration changes in inflammatory and epithelial markers (IL-1a, IL-6, sEcad, MMP-9, IL-17) but not chemokines (MIP-1b, MIP-3a, MIG) were inversely associated with their pre-sex absolute concentrations. Unsupervised hierarchal clustering identified two distinct participant groups based on pre-sex soluble immune factor concentrations; participant clustering was significantly associated with genital inflammation, defined using a combinatorial score of HIV-associated markers, but was not associated with condom use. We conclude that the preexisting vaginal immune milieu is a strong determinant of the inflammatory effects of sex, where participants with baseline cervicovaginal inflammation experience a reduced inflammatory response to sex but are more likely to experience elevated HIV-associated markers beyond 72 hrs post-sex. Our findings suggest biological factors that may contribute to global discrepancies in female HIV-acquisition risk, highlighting the need for population-specific HIV prevention strategies and treating the vaginal immune milieu in populations where vaginal inflammation is common.

13

From data providers to data leaders: developing community-led quantitative research capacity using HIV/STI program data in Kenya

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Background: Despite progress in participatory HIV/STI research overall, meaningful community leadership in quantitative research remains limited. Gaps are especially evident in studies with data routinely collected from HIV/STI health program data. The HEKA initiative was established by seven community-based organizations implementing HIV/STI prevention programs among gay, bisexual, and other men who have sex with men in Kenya. HEKA aimed to shift community roles from mere data providers to active leaders and users of quantitative data.

Methodology: HEKA's guiding framework combined principles from the Greater and Meaningful Involvement of People Living with HIV/AIDS and Community-based Program Science. Twenty-one community members, primarily program managers and monitoring and evaluation staff, collaboratively engaged in three iterative capacity-building in-person workshops focusing on quantitative research skills, with virtual engagement between workshops. Academic partners supported community teams through structured mentorship, co-developed skills development sessions, and training in R programming. Together, we cleaned and harmonized the routine program data to set the foundation to address community-defined questions and support program delivery.

Results: Through sustained capacity-building activities, community researchers developed essential quantitative research competencies. These included data management, analysis, visualization, and scientific communication. Community partners successfully led the cleaning of 6 years of program data and preliminary analyses that generated actionable insights to guide HEKA's research strategy. A critical reflection highlighted misalignment between funder-imposed program indicators and local priorities, advocating for the creation of community-defined metrics. Community partners also developed stronger confidence and leadership in identifying research questions and using quantitative evidence.

Conclusions/Significance: HEKA demonstrates the feasibility and substantial value of authentic community leadership in quantitative HIV/STI research. Empowering communities with quantitative research skills has the potential to enrich program relevance and for communities to independently seek research funding. This transformative model offers a framework for shifting research paradigms, underscoring that communities can—and should—lead quantitative research processes.

14

Structure-guided 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 ~60% 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 sought to optimize the GW scaffold through a structure-guided approach. 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 two 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. Overall, this study highlights the potential of targeting Yck2 as an antifungal strategy to combat systemic mycotic infection.

15

Rapid identification of filamentous fungi using matrix- assisted laser desorption/ionization time- of -flight mass spectrometry (MALDI - TOF MS)

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OBJECTIVE: Rapid and accurate identification of filamentous fungi remains challenging in a clinical microbiology laboratory. Our study evaluated the performance of MALDI - TOF MS for identification of filamentous fungi isolated from clinical specimens.
METHODS: We conducted a retrospective study including 135 isolates of filamentous fungi which are commonly encountered in clinical specimen. The isolate was cultured on IMA and SAB-CG plates in parallel. The growth at day 3 and 7 was subject to MALDI-TOF-MS analysis using the Mycelium Transfer (MyT) extraction technique. Briefly, MyT technique involves picking up the growth from the edge of the colony in presence of formic acid with a sterile toothpick, followed by spotting with matrix on the target plate for MALDI analysis against Bruker's filamentous fungi library. We calculated the accuracy of MALDI identification by MyT technique to both species and genus level in comparison to the reference ID from Public Health Ontario Laboratory. We also analyzed the impact of different culture media and incubation period on accuracy of MALDI identification.
RESULTS: Overall, the accuracy is 93.5% at least to the genus level and 73.5% to the genus and species level. Superior performance was among Aspergillus spp isolates 93% identification to genus and species level and remaining 7% had genus level identification. Similar performance was observed among Mucorales. IMA media are superior to SAB-CG with accuracy to genus level at least with 87.5% compared with SAB-CG under 73.5%. We observed superior performance with growth at 3 days of incubation with accuracy of 92% compared to 29% on day 7 of incubation to genus level identification.
CONCLUSION: MALDI analysis with MyT technique provides accurate identification on commonly encountered filamentous fungi in a clinical microbiology laboratory. It also exhibits advantage of early identification on young culture while morphological features are still developing for conventional identification.

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In-depth T-cell phenotyping and its association with relapse in transplant recipients with human Cytomegalovirus (HCMV) DNAemia

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It is estimated that 20–30% of solid organ transplant (SOT) recipients with cytomegalovirus (CMV) DNAemia relapse despite antiviral therapy. Relapse increases the risk of disease, allograft rejection, antiviral resistance, and mortality. We aimed to perform an in-depth analysis of T-cell subsets associated with clinical CMV relapse, focusing on exhaustion, activation, and effector markers in CD4+ and CD8+ T-cells in peripheral blood. Blood samples were collected from 33 SOT recipients at the onset of CMV DNAemia and again at 4 weeks. A 25-colour multiparameter flow cytometry panel was used to identify key subsets among global and CMV-specific T-cells. Relapse was defined as a plasma viral load >1,000 IU/mL after initial clearance, within six months of the initial DNAemia episode. The median age of participants was 59.0 years [23.0–76.0]. The median time from transplant to DNAemia onset was 255 days [22–7591]. Thirteen participants (39.4%) developed CMV relapse. CMV-specific T-cell responses were low across the cohort and did not differ significantly between relapsing and non-relapsing participants. However, relapsing recipients had significantly lower frequencies of granzyme B+ CD4+ (p=0.009) and CD8+ (p=0.038) T-cells at onset, and lower CD154+ CD4+ T-cells at 4 weeks (p=0.003). Exhaustion profiling revealed that at DNAemia onset, relapsing recipients had fewer CD4+ (p=0.025) and CD8+ (p=0.004) T-cells with the CD160−CTLA-4−LAG-3+PD-1+TIGIT+TIM-3− phenotype. By week 4, these recipients had higher frequencies of CD8+ T-cells with a CD160−CTLA-4+LAG-3−PD-1−TIGIT+TIM-3− phenotype (p=0.044). Granzyme B, CD154, and exhaustion marker profiles on global T-cells may serve as biomarkers for CMV relapse risk. These findings underscore the roles of T-cell activation, cytotoxicity, and exhaustion in CMV relapse and support the development of targeted immunomodulatory interventions.

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Novel DNA glycosylase-based enzyme restricts phage replication in *Vibrio parahaemolyticus*

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With rising antimicrobial resistance (AMR) posing a serious global health threat, phage therapy is emerging as a viable alternative to antibiotics. However, the dynamic and complex interplay between phages and bacterial hosts complicates therapeutic applications. Bacteria have evolved diverse anti-phage defence mechanisms through an evolutionary arms race with phages, yet many of these defences remain uncharacterized. Understanding these mechanisms is essential for optimizing phage therapy by engineering phages capable of bypassing bacterial defences and effectively targeting specific pathogens.

In this study, we investigate a novel anti-phage system, VP1840, from a clinical strain of Vibrio parahaemolyticus. Bioinformatic analyses and structural predictions reveal that VP1840 resembles DNA repair enzymes in the Helix-hairpin-Helix DNA glycosylase superfamily, which excise modified bases in DNA. Here, we demonstrate that VP1840 functions as a DNA glycosylase- and lyase-like enzyme, selectively target guanine base modification to inhibit phage replication. VP1840 is conserved across multiple taxa, suggesting it may play a widespread role in phage resistance among bacteria. By characterizing VP1840 and similar anti-phage systems, this research contributes to our understanding of an emerging bacterial defence strategy and provides insights critical to advancing phage therapy.

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SLAM - a molecular multipurpose tool for diagnostic innovations

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The Surface Lipoprotein Assembly Modulator (SLAM) is a Gram-negative bacterial outer membrane protein that enables highly specific translocation of substrates from the periplasmic face of the lipid bilayer onto the extracellular surface. I have engineered different SLAM homologs to function in Escherichia coli using a single vector backbone that can be subcloned in two different ways for a diverse range of functionality: (1) tethering SLAM substrates for surface-display, or (2) secreting SLAM substrates directly into the culture supernatant as exoproteins. As a surface-display system, my engineered constructs can be used to probe functionality of any matching SLAM/substrate pairs. These tethered surface substrates can also be used to display other protein epitopes in fusion. As a secretion system, we can uncouple normally tethered SLAM substrates and secrete them into the culture supernatant. We achieved secretions yields up to 50 milligram per liter of culture, improving upon conventional purification methods of these substrates by 20 to 30-folds. The secreted proteins maintain their conformational fold and function in binding to their cognate substrate. The secreted substrates can also be engineered in fusion to other proteins, highlighting its potential as a protein-cargo production platform. The SLAM secretion system simplifies purification by eliminating downstream processing steps and thereby significantly lowering production costs of biologics.

To further the development of this system, I am combining in-silico designs of protein binding epitopes from Generative AI and grafting these "minibinders" onto our SLAM secreted substrates. Conventional molecular diagnostics rely heavily on monoclonal antibodies, which have long development cycles and complicated production methods. I am leveraging the cost-effective production capabilities of my engineered SLAM system to change how we can develop new molecular diagnostics to quickly adapt to emerging and rapidly evolving pathogens.

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Therapeutic Efficacy of INT131 in an EcoHIV Mouse Model of HIV-Associated Neurocognitive Impairment

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Background: HIV-associated neurocognitive impairment (NCI) affects approximately 50% of individuals living with HIV, leading to cognitive, motor, and behavioral deficits. While activation of peroxisome proliferator-activated receptor gamma (PPARγ) has demonstrated anti-inflammatory effects in neurodegenerative disorders, its role in HIV-related NCI and potential sex differences in its efficacy remain unclear. This study investigated the therapeutic potential of INT131, a selective PPARγ agonist, in mitigating HIV-induced brain inflammation and cognitive impairment in an EcoHIV mouse model.

Methods: Male and female C57BL/6 mice were injected intraperitoneally (IP) with either saline or EcoHIV (4×10^6 pg/ml of p24) and assessed six weeks post-infection. The experimental groups included: (i) non-infected control, (ii) non-infected + INT131 (20 mg/kg/day, IP osmotic pump for 28 days), (iii) EcoHIV-infected, and (iv) EcoHIV-infected + INT131. Molecular analyses of brain and spleen tissues were conducted using qPCR, western blot, and immunohistochemistry to assess viral gene expression, inflammatory markers, oxidative stress, NF-κB signalling, PPARγ expression, and blood-brain barrier (BBB) integrity. Behavioural assessments examined locomotion, learning, memory, and anxiety-like behavior.

Results: INT131 treatment significantly reduced viral gene (vif and tat) expression, viral p24 protein levels, and inflammatory cytokines/chemokines (TNF-α, IL-1β, IFN-γ, and CCL2) and oxidative stress markers (Nos2 and Hmox1) in the brain and spleen of both male and female infected mice. PPARγ expression, neuroprotective markers (NeuN and Syp) and BBB tight junction proteins (Cldn5, Ocln, and Tjp1) were restored in the brain cortex. Behavioral deficits in EcoHIV-infected mice, including impairments in locomotion, learning, memory, and heightened anxiety-like behavior, were reversed following INT131 treatment. Additionally, INT131 significantly reduced phosphorylated Nf-κB in the brain cortex.

Conclusion: PPARγ activation via INT131 may serve as a novel therapeutic strategy for HIV-associated brain inflammation and BBB dysfunction, providing a foundation for the clinical translation of INT131 in the treatment of HIV-associated NCIs. (Supported by CIHR).

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Integrons are anti-phage defence libraries in the Zoonotic Pathogen *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus is a zoonotic pathogen and the leading cause of seafood-borne gastroenteritis in humans. Growing antibiotic resistance against Vibrio species is creating significant problems for treatment of these infections in both contexts. One promising alternative is to use bacterial viruses, known as phages, to target and destroy these pathogens in aquaculture prior to human consumption. However, bacterial encode a variety of mechanisms to defend themselves from phage infection, known as anti-phage defence. Uncovering how bacteria do this will allow us to develop ways to counter these defences prior to phage therapy.

Anti-phage defence genes often cluster in genomic regions known as defence islands. To identify the global arsenal of antiphage defence in this pathogen, we used DefenseFinder to map known anti-phage defence genes across available complete genomes of this organism. By clustering neighbouring genes and identifying those commonly associated with defence, we identified that known anti-phage defence genes were frequently found within large genomic regions called sedentary chromosomal integrons (SCI) – genetic elements that capture and store gene cassettes. Although SCIs have long been recognized for their role in bacterial adaptation, their involvement in phage defence had not been previously described.

After verifying that many of the identified systems conferred genuine anti-phage defence, we explored whether additional defence genes might reside within SCIs. By cloning 57 gene cassettes from the chromosomal integron of the pandemic V. parhaemolyticus strain (RIMD 2210633) and screening them against both the BASEL collection in E. coli and an in-house phage collection active against V. parahaemolyticus, we discovered nine previously unrecognized defence systems. Our findings reveal that integrons serve as a critical reservoir of anti-phage defence. Given the ancient origin of integrons and their broad distribution among Proteobacteria, this work offers a promising approach for discover new anti-phage defence mechanisms across diverse bacterial species.

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Monkeypox virus shedding despite tecovirimat treatment in a cohort in Toronto, Canada

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Tecovirimat (TPOXX) is an antiviral authorized for the treatment of mpox infections in Canada, but recent clinical trials found it has no impact on symptom duration. We conducted a prospective cohort study of individuals diagnosed with mpox in Toronto, Canada. Skin lesion swabs were collected weekly to quantify infectious monkeypox virus (MPXV) shedding through cell culture. The presence of antiviral resistance mutations was assessed by PCR and sequencing the F13L gene. Among 17 participants, 9 received tecovirimat, with a median initiation time of 14 days post-symptom onset. Infectious MPXV was detected in 31% (17/55) of lesion swabs from tecovirimat-treated participants and 32% (20/62) from untreated individuals. Shedding kinetics were similar between groups, with persistent infectious virus detected in several participants beyond two weeks of symptoms. In skin lesion samples collected more than 7 days after tecovirimat initiation, four treated participants still shed viable virus, including up to 15 days after tecovirimat initiation. No known resistance mutations were identified in viral sequences from a subset of lesion swabs from both treated and untreated individuals, suggesting that tecovirimat resistance mutations were not widely circulating in Toronto during the 2022 outbreak. Our findings suggest that tecovirimat does not significantly impact the duration of infectious MPXV shedding from skin lesions, aligning with recent randomized trial results. These findings highlight the need for alternative antiviral strategies and continued genomic surveillance to monitor resistance emergence.

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Characterizing pathogenicity differences among *Gardnerella* in the vaginal microbiome

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Bacterial vaginosis (BV) affects 1/3 of reproductive-aged women and is characterized by a shift from Lactobacillus bacteria dominance to depletion in the vaginal microbiome, leading to an overgrowth of diverse anaerobic bacteria. Gardnerella bacteria is strongly associated with BV; they directly adhere to the vaginal epithelium and are cytotoxic. Interestingly, Gardnerella are present in the vaginal flora of healthy women, though with significantly lower abundance. Whether there are differences between Gardnerella strains among women with BV or between BV versus non-BV microbiomes is not known. The Gardnerella genus of bacteria is diverse; subdivided into 4 genetically similar sub-groups (clades) each comprised of multiple species. I aim to explore pathogenicity differences among Gardnerella to improve our understanding of Gardnerella biology and its contribution to BV. To examine differences in pathogenicity among Gardnerella, I created a Gardnerella collection by isolating and culturing bacteria from the vaginal samples of Kenyan women. Using PCR-based methods, I have identified Gardnerella isolates and categorized them into their respective clades. My current collection of Gardnerella isolates contains representatives from all 4 clades. I have observed distinct Gardnerella diversity patterns between different microbiome types. Normal vaginal microbiomes are dominated by one clade of Gardnerella whereas multiple clades of Gardnerella colonize BV vaginal microbiomes. I am currently investigating clade differences among Gardnerella in three pathogenicity features: i) adherence to vaginal epithelial cells, ii) cytotoxicity effect on vaginal epithelial cells, and iii) resistance to metronidazole, a BV antibiotic therapeutic. Preliminary experiments illustrate Gardnerella clade differences in metronidazole resistance and induction of cytotoxicity on vaginal epithelial cells. These results indicate that different clades potentially drive unique aspects of BV pathogenesis. Continuation of experiments surveying more Gardnerella isolates will aid in strengthening our understanding of the role Gardnerella diversity plays in BV etiology, leading to improved prevention and treatment.

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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.

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Safety and immunogenicity of adjuvanted subunit respiratory syncytial virus (RSV) vaccination in high-risk transplant recipients.

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UHN

Purpose: Lung transplant (LT) and allogeneic hematopoietic cell transplant (alloHCT) recipients are at increased risk of severe lower respiratory tract disease and mortality from RSV infection. Immunocompromised populations were excluded from the published clinical trial of the approved adjuvanted RSV vaccine, RSVPreF3 (Arexvy, GSK). To address this evidence gap, we evaluated the safety and immunogenicity of RSVPreF3 in LT and alloHCT recipients.

Methods: We conducted a non-randomized, open label, interventional study at the University Health Network (NCT #06593210). Stable outpatients ≥3 months post-LT or ≥6 months post-alloHCT received a single dose of RSVPreF3. Blood was collected at baseline and 4 weeks post-vaccination. Anti-prefusion F IgG levels were measured using electrochemiluminescence immunoassay, with seroconversion defined as a ≥4-fold increase in antibody level. RSV-specific T-cell responses were assessed via flow cytometry following overnight peptide stimulation of peripheral blood mononuclear cells (PBMCs) and identification of antigen-specific polyfunctional T-cells. Safety was evaluated through participant symptom diaries and standardized interviews on days 7 and 42.

Results: A total of 86 participants (40 LT, 46 alloSCT) were enrolled. The vaccine was well tolerated; no rejection or GVHD events occurred. Anti-prefusion IgG levels increased significantly in both groups (LT: p=0.0087; alloSCT: p=0.0030), with median fold-changes of 1.4 (LT) and 2.1 (alloSCT). Seroconversion was observed in 37% of LT and 29% of alloSCT recipients. In alloSCT, seroconversion was associated with a longer time from transplant (p=0.016). RSV-specific CD4+ T-cell responses increased in both groups (LT: p=0.018; alloSCT: p=0.0002), while CD8+ T-cells increased only in LT (p=0.016). At 4 weeks, 72% (LT) and 81% (alloSCT) had detectable CD4+ T-cells; 62% and 52%, respectively, had detectable CD8+ T-cells.

Conclusion: The RSVPreF3 vaccine was safe and elicited robust CD4+ T-cell responses in both lung and alloHCT recipients, although seroconversion rates were modest. Given the importance of antibodies in protection against RSV, strategies to enhance humoral immunity in transplant recipients are warranted. Alternate strategies to enhance humoral immunity, including a second dose of vaccine, warrant further investigation.

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Virus-inclusive Single-cell RNA Sequencing Reveals Broad Cellular Tropism and Common Pathway Dysfunctions Linked to Cytomegalovirus Infection in Organ Transplant Recipients

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UHN

Introduction: Cytomegalovirus (CMV) causes significant morbidity and mortality in transplant recipients and is traditionally thought to target CD14+ monocytes and select cell types. However, its full tropism and pathogenesis in transplantation remain poorly understood. Prior studies relied on invitro models and lab-adapted strains, limiting insights into in vivo infection dynamics.

Methods: We performed virus-inclusive single-cell RNA sequencing (scRNA-seq) on PBMC from 4 transplant recipients (3 lung, 1 kidney-pancreas)with high CMV DNAemia (>10^5 IU/mL) to simultaneously profile viral and host transcripts. The median age of participants was 48 years, and blood was collected 1.5 days post-DNAemia diagnosis. A total of 20,356 single cells, pooled from the 4 participants, passed quality control. Cell types and transcript levels were computationally identified; transcriptional changes in CMV-positive cells were analyzed using the REACTOME pathway database.

Results: 99 unique CMV transcripts were detected across 92% of all PBMC subsets, spanning diverse hematopoietic lineages (Fig 1). In all, only 1.7% of cells harboured viral transcripts, with the highest frequency in hematopoietic stem and progenitor cells (HSPCs), followed by subsets of NK cells, B-cells, and neutrophils. HSPCs exhibited the broadest viral transcript expression across all viral life cycle phases, suggesting productive infection. >250immune and metabolic pathways were significantly altered in CMV-positive cells, including downregulation of host protein synthesis, mitochondrial function and energy metabolism, and cell signalling pathways. In secondary scRNA-seq datasets from three additional lung transplant recipients with paired PBMC and lung biopsy samples but no detectable CMV DNAemia, CMV transcripts were absent despite high-risk donor serostatus, suggesting viral latency may involve low-level transcription requiring additional enrichment methods.

Conclusions: We demonstrate broad CMV cellular tropism in blood, with HSPCs as key targets. Widespread immune, protein synthesis, and metabolic dysregulation across infected cells highlights CMV's role in immune dysfunction in transplant recipients. These findings establish virus-inclusive scRNA-seq as a powerful tool for dissecting viral pathogenesis and provide critical insights to inform antiviral and immune-based therapeutic strategies in transplantation.

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Guarding the goods: Nutrient sequestration as a competitive strategy in *Streptomyces*

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Microbial competition for nutrients is inevitable in most environmental and host-associated ecosystems. In this study, we use the soil-dwelling bacterium Streptomyces venezuelae to explore how microbes compete over recalcitrant carbon and nitrogen sources. We show that S. venezuelae can efficiently grow on raw, insoluble chitin as its sole carbon and nitrogen source, driven by a complex chitinolytic system and the specialized chitobiose importer DasABC. Loss of this importer impairs growth and leads to the accumulation of extracellular chitobiose, which subsequently enables growth of the non-chitinolytic Bacillus subtilis in co-culture. This demonstrates that S. venezuelae not only degrades chitin but also safeguards the resulting oligomers released by saprophytic digestion from competitors.
While S. venezuelae is not a classical pathogen, this work reveals a fundamental microbial strategy with broad relevance: Control of shared nutrient pools as a mechanism of ecological advantage. By highlighting how extracellular digestion creates both opportunity and risk, our findings underscore a principle increasingly appreciated in infectious disease biology — that access to nutrients can be as decisive as toxin production in microbial survival and dominance.

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Quorum sensing molecules vs virulence: Detecting farnesol and tyrosol in *Candidozyma auris* biofilms

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Candidozyma auris is an emerging multidrug-resistant yeast described in 2009 and is frequently associated with nosocomial outbreaks. Since the description, research has been conducted on its underlying biological features, particularly quorum sensing molecules (QSMs) such as farnesol (Far) and tyrosol (Tyr). All of the data in the literature show QSMs detection and quantification in planktonic cultures. With this in mind, our main goal was to quantify Far and Tyr in C. auris, design a biofilm model, and understand their role in virulence using the in vivo Galleria mellonella larvae model. Using C. albicans SC5314 as comparison and C. auris strains that represent four clades (CBS 12766 – clade I; CBS 10913 – clade II; B11221 – clade III and B11244 – clade IV), C. albicans showed the higher total amount of Far (260.78±55.34 µg) and Tyr (164.40±54.78 µg) quantified by gas chromatography coupled with mass spectrometry (GC-MS), but also higher biofilm biomass and biofilm dry weight. With relativized data per biofilm dry weight (QSM production per µg of biofilm), the scenario changes. The most virulent C. auris CBS 12766 produced the highest QSMs levels, while the lowest virulent strain CBS 10913 produced the lowest values among C. auris strains. Notably, all C. auris strains exhibited higher QSMs production per biofilm dry weight and virulence data associated with greater QSMs levels and fungal burden. In contrast, the reference strain C. albicans SC5314 produced greater QSMs and higher biofilm total biomass but had a lower capacity to produce the same amount of Far and Tyr per biofilm µg and fewer cell counting in the infected larvae. Thus, we described Far and Tyr in C. auris biofilms, suggesting a relation between QSMs production and virulence.

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Genomic epidemiology, phylogenetic, and phylodynamic analysis of respiratory syncytial virus in the Greater Toronto Area, 2014-2023

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Respiratory syncytial virus (RSV) groups RSV-A and RSV-B are a significant cause of respiratory tract infections in children and older adults. RSV cases and hospitalizations significantly decreased in the beginning of the COVID-19 pandemic due to non-pharmaceutical interventions (NPIs), however earlier than usual surges were observed following the easing of COVID-19 lockdown measures. We aim to 1) identify RSV-A and RSV-B lineages that circulated from 2014 to 2023 in Ontario, Canada, 2) reconstruct the phylogenetic relationships of circulating lineages based on whole genome and G gene sequences, and 3) characterize subgroup specific evolutionary and epidemic parameters using phylodynamic approaches. Retrospective RSV samples (n=~600, 2014 to 2023) collected from the Greater Toronto Area were subjected to whole genome sequencing (WGS). Consensus genomes were generated and assigned to Nexclade lineages. Phylogenetic trees were constructed using MAFFT, IQTree, and the Nextstrain and Augur pipelines. Phylodynamic modelling was performed using the BEAST2 BDSKY serial model. Quality consensus genomes meeting 100x depth of coverage and 75% breadth of coverage were generated for 152 RSV-A and 186 RSV-B samples. A total of 11 RSV-A A.D and 5 RSV-B B.D lineages were identified with distinct A.D lineages (A.D.1.7, A.D.2.1, A.D.5.2, A.D.2.1, A.D.1.8) and B.D lineages (B.D.E.1) only appearing post-pandemic. Whole genome-based phylodynamics found that the time to the most recent common ancestor (TMRCA) for RSV-A A.D was around 2008.13 95% HPD interval [2007.15, 2009.32]. The evolutionary rate estimate for RSV-A A.D based on the BDSKY model and a prior rate of 4.42E-4 was 6.64E-4 95% HPD interval [5.32E-4, 8.02E-4]. These findings reveal specific RSV-A A.D and RSV-B B.D lineages and respective epidemic behaviour associated with the increase in cases following the removal of COVID-19 restrictions. We show that RSV in circulation following the removal of COVID-19 pandemic measures are distinct from pre-pandemic lineages and epidemic patterns.

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Virus-inclusive Single-cell RNA Sequencing Reveals Broad Cellular Tropism and Common Pathway Dysfunctions Linked to Cytomegalovirus Infection in Organ Transplant Recipients

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Cytomegalovirus (CMV) causes significant morbidity and mortality in transplant recipients and is traditionally thought to target CD14+ monocytes and select cell types. However, its full tropism and pathogenesis in transplantation remain poorly understood. Prior studies used in vitro models and lab-adapted strains, limiting insights into in vivo infection dynamics. We performed virus-inclusive single-cell RNA sequencing (scRNA-seq) on PBMCs from four transplant recipients (three lung, one kidney-pancreas) with high CMV DNAemia (>10^5 IU/mL) to simultaneously profile viral and host transcripts. The median participant age was 48 years, and blood was collected 1.5 days after DNAemia diagnosis. A total of 20,356 single cells pooled from all participants passed quality control. Cell types and transcript levels were computationally identified, and transcriptional changes in CMV-positive cells were analyzed using the REACTOME pathway database. Ninety-nine unique CMV transcripts were detected across 92% of PBMC subsets, spanning diverse hematopoietic lineages. Overall, 1.7% of cells harbored viral transcripts, with the highest frequency in hematopoietic stem and progenitor cells (HSPCs), followed by subsets of NK cells, B cells, and neutrophils. HSPCs showed the broadest expression of viral transcripts across all life cycle phases, suggesting productive infection. More than 250 immune and metabolic pathways were significantly altered in CMV-positive cells, including downregulation of host protein synthesis, mitochondrial function, energy metabolism, and cell signaling. In secondary scRNA-seq datasets from three additional lung transplant recipients with paired PBMC and lung biopsy samples but no detectable CMV DNAemia, no CMV transcripts were detected, suggesting that viral latency may involve low-level transcription requiring enrichment methods. We demonstrate broad CMV tropism in blood, with HSPCs as key targets. Widespread transcriptional dysregulation across infected cells underscores CMV's role in immune dysfunction and highlights virus-inclusive scRNA-seq as a powerful tool for studying viral pathogenesis in transplantation.

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Microbial Transmission via Medical Gloves: Investigation Touch-Contact Pathways in Clinical Settings

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Touch transmission of pathogens is a significant contributor to the spread of infectious disease within hospitals, primarily through healthcare workers interacting with patients and their surroundings. These procedures typically involve the use of medical gloves to prevent cross contamination, though several studies have highlighted the contamination potential and risk that medical gloves play in microbial transmission. Our work focuses on understanding the aspects of commercial medical glove surfaces which mediate this transmission and modifying medical glove surfaces with novel superhydrophobic microtopographies which can reduce microbial transmission. Superhydrophobic post and groove topographies with critical dimensions between 2.5 to 30 um were evaluated through controlled touch contract experiments with 6 microbially relevant species. Following compressive touch contact testing, our results show a 1.5 – 2.5 log reduction in the transfer of microbial cells to the glove surface. The impact of these modifications and mechanism by which this reduction occurs was investigated through sequential touch contact testing for Staphylococcus aureus and Klebsiella pneumoniae to several fomite surfaces (stainless steel, glass, ABS). Significant reductions to the transfer efficiencies (1.75 – 2.41% for modified gloves compared to 39.1 – 43.6% for conventional gloves) and the physical mechanisms for microbial transmission were determined based on these experiments. Lastly, we focused on preliminary evaluation of these modified medical gloves in clinical environments through assessing transmission from common fomite surfaces in the rooms of discharged patients. The preliminary results of these studies highlight the importance of surface hydration on microbial transmission, and the future design directions of microtopographies for success in clinical environments. Overall, our work highlights the aspects of commercially available medical gloves which contribute to microbial transmission, and the impact that altering the surface with superhydrophobic microtopographies can have on reducing microbial transmission in clinical settings.

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Investigating the differential vulnerability of microglia and astrocytes to HHV-6A exposure, and the impact of selective infection on neuronal function

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Background: Human herpesvirus 6, or HHV-6, is a widespread virus that infects over 90% of the global population by the age of two. HHV-6 is becoming increasingly linked to neurological conditions, including epilepsy, encephalitis, and chronic diseases such as multiple sclerosis. However, understanding its effects on the central nervous system (CNS) is challenging due to the limited availability of primary human samples.
Methods: To better understand the effects of infection in the CNS, we leverage stem cell engineering to describe HHV-6A infection *in vitro*. We differentiated human induced pluripotent stem cells into neurons, microglia, and astrocytes, forming both 2D monocultures and 3D immune-competent cortical spheroids, followed by exposure to HHV-6A. We used RNA sequencing to uncover activated pathways in each cell type, live imaging to characterize microglial death, and LDH assays to measure lytic cell death. We also performed immunofluorescent staining to determine viral neurotropism, and multi-electrode array recordings to assess neuronal firing patterns.
Results:Our results showed that HHV-6A efficiently infects all three CNS cell types. In microglia, we observed a significant 95% drop in viability, along with reduced movement speed and displacement. Astrocytes, on the other hand, showed decreased proliferation during acute infection, but continued to harbor the virus 30 days later and formed syncytia, suggesting they may serve as viral reservoirs. Neurons displayed disrupted network synchronicity, but individual neurons became more rhythmic and burst-prone after infection. Importantly, HHV-6A activated interferon response genes like IFI6 and IFITM3 across both 2D and 3D models, pointing to a strong inflammatory signature.
Conclusion: Microglia and neurons are highly vulnerable to HHV-6A induced dysfunction, while astrocytes sustain chronic infection and may enable viral integration and potential reactivation. These insights help us better understand HHV-6A's effects on the brain and may inform therapeutic strategies to protect against infection or suppress reactivation.

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HIV-1 Unlocks a Non-Classical Mode of Viral Entry in CD4 Negative Cells

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Despite improvements in HIV-1 therapies drug treatment failure and the inability to target latent viral reservoirs remain major limitations. Infection generally requires HIV recognizing the primary receptor CD4, followed by binding to either of the chemokine coreceptors, CXCR4 or CCR5. This binding mode enables cell entry by HIV gp41 fusion with the cell membrane. Investigators have believed for some time that initial binding to CD4 is required for HIV-1 infection. However, studies have shown that HIV-1 can enter cells lacking CD4 although this remains controversial, and the mechanisms are unclear. This study aims to identify a model CD4-negative cell line that is infectable with HIV-1 to study alternate entry mechanisms and determine whether CD4-negative cells can act as latent viral reservoirs. CD4-negative human osteosarcoma (HOS) cells expressing either coreceptor were infected with an envelope-pseudotyped, luciferase or GFP reporter HIV-1; JR-FL-envelope for CCR5+ and HXB2-envelope for CXCR4+ cells. These cells were confirmed to be CD4 negative through flow cytometry, mRNA expression & the use of a CD4 blocking antibody (Ibalizumab) during infection assays. We found that HIV-1 can infect HOS cells, as demonstrated by post-infection luciferase and GFP expression that was blocked by the integration inhibitor Raltegravir. Infection in HOS cells is resistant to Ibalizumab but susceptible to coreceptor blockers, suggesting that infection is CD4 independent but coreceptor dependent. These results suggest that in the absence of the CD4 receptor, HIV-1 uses an alternative mechanism for viral entry into HOS cells. Further studies will examine infected GFP-positive cells, including single-cell RNA sequencing, determining mechanisms used for viral entry, and the use of a red/green reporter virus to investigate latency. Deepening our understanding of viral entry into CD4-negative cells may help identify unknown viral reservoirs, improve HIV-1 therapeutics, and contribute to finding an eventual cure.

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Determining the role of neural progenitor microRNAs during Zika virus infection

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Zika virus (ZIKV) is a rapidly emerging mosquito-borne virus. ZIKV arrived in the Americas for the first time in the 2015 Brazilian outbreak. During this unprecedented outbreak, congenital microcephaly cases in Brazil surged approximately 10-fold. Microcephaly, a birth defect where a baby's head is smaller than expected for the given age and sex, is associated with complications including seizures, developmental delay, and hearing loss. Previous ZIKV outbreaks were not linked to congenital birth defects, but it is now widely accepted that ZIKV infection during early pregnancy can cause microcephaly. ZIKV preferentially infects neural progenitor cells (NPCs), crucial precursors that differentiate into various neural cell types during development. ZIKV infection of NPCs reduces growth and increases cell death. Infection can also disrupt microRNA (miRNA) processing and expression. miRNAs are small RNAs that alter gene expression and regulate critical checkpoints in NPCs and embryonic brain development. We hypothesize that ZIKV may contribute to microcephaly by misregulating important miRNAs that coordinate neural development. While previous studies have shown that ZIKV infection can disrupt cellular miRNA expression, the mechanisms by which ZIKV modulates miRNAs remain unknown. We uncovered direct ZIKV interactions with two NPC-specific miRNAs essential for embryonic brain development. We are now generating ZIKV mutant infectious clones in which the binding sites for these neuroprotective miRNAs are disrupted, and will infect NPCs with these mutants to understand how the specific abrogation of these interactions affects NPC health and ZIKV replication. This work will elucidate the functional impacts of these interactions in the fetal brain and expand the tools available for studying ZIKV-miRNA interactions in vivo.

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Multi-omics analysis of the maternal gut microbiome reveals functional changes during pregnancy

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Background: The gut microbiome is critical to human health. Host factors such as genetic predisposition, diet, and pathogen exposure can disrupt the gut microbiome, causing dysbiosis. Dysbiosis is implicated in many diseases, including obesity and undernutrition. During pregnancy, the gut microbiome undergoes significant changes to meet increased metabolic demands. Previous studies of the gut microbiome have largely focused on bacteria and neglected the role of eukaryotic microbes. Understanding the contributions of protists to the maternal gut microbiome will improve our understanding of host:microbiome interactions.

Methods: This project investigates the influence of protists on gut microbiome function during pregnancy. Stool samples were collected in the first and third trimesters of pregnancy from an established cohort of 150 women in Pakistan. Metatranscriptomics profiling was conducted to assess differences in microbial gene expression between trimesters. Metabolomics analysis was performed to quantify the concentrations of 638 metabolites, providing insights into changes in metabolite production and biochemical activity in the gut during pregnancy.

Results: Metatranscriptomics analyses identified the presence of the protist Giardia in some samples. Third-trimester samples demonstrated increased expression of genes involved in energy and carbohydrate metabolism. Metabolomic data showed trimester-specific shifts in metabolite profiles. These results suggest changes in gut microbiome function and metabolite production across pregnancy, highlighting the need for further investigation into how protist colonization may influence the host microbiome during pregnancy.

Conclusions: This work is the first to systematically explore the interactions between protists and the maternal gut microbiome. These findings reveal changes in microbial community function between the first and third trimesters of pregnancy. Next steps include performing 18S ribosomal RNA sequencing to more extensively profile eukaryotic microbes in stool and using a mouse model to investigate the impact of dietary interventions on protist colonization. This research may inform the development of microbiome-based dietary interventions to prevent infection with parasitic protists.

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Genomic approaches improve *Treponema pallidum* lineage classifications and identifies antimicrobial resistance patterns

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Syphilis cases have increased dramatically since its near elimination in the late 1990s. This resurgence, along with increasing rates of macrolide resistance and congenital syphilis, has triggered renewed efforts to better understand and control the disease. We analyzed 809 T. pallidum genomes and created a new genome-based hierarchical lineage framework, recapitulating the major T. pallidum lineages and characterizing sub-lineages. An updated pangenome was constructed, revealing that T. pallidum subsp. pallidum lineages are determined by a single hypothetical major outer sheath C-terminal domain-containing gene while no significant genetic difference was observed between T. pallidum subsp. pertenue and T. pallidum subsp. endemicum. A scan for antimicrobial resistance in T. pallidum determined that macrolides may still be effective in specific geographic regions. Notably, no resistance to doxycycline or penicillin was identified. This study introduces a new approach to characterize T. pallidum and highlights the significance of pangenomes in supporting public health.

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Functional and structural characterization of monoclonal antibodies for protection against pathogenic *Neisseria* species

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Neisseria meningitidis (Nme) and Neisseria gonorrhoeae (Ngo) pose significant health threats globally. Despite available vaccines targeting several serogroups of Nme, emerging strains and vaccine coverage gaps continue to challenge control efforts. Ngo has developed alarming
resistance to multiple antibiotics, complicating treatment and raising concerns about untreatable infections. Both pathogens underscore the critical need for novel treatment strategies and vaccine development to mitigate their public health impact. Here we describe the discovery of
monoclonal antibodies directed against the nutritional receptor TbpA that were cross reactive against both Nme and Ngo strains and were protective against infection in vivo in a mouse Nme sepsis model. One antibody, designated 3A3, exhibited exceptional attributes including TbpA functional neutralization and induction of opsonophagocytosis by macrophages. We have also delineated the structural basis for 3A3 activity using CryoEM to fully describe the potential clinical utility of this antibody.

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Phylodynamic modeling of Seasonal Respiratory Viruses: Influenza A and Respiratory Syncytial Virus circulated in 2023-24, Ontario, Canada

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Influenza viruses and Respiratory Syncytial Virus (RSV) are among the leading causes of seasonal respiratory illness in Ontario, Canada. Although their circulation was disrupted during the COVID-19 pandemic, recent seasons have seen a resurgence, particularly in RSV, placing a significant burden on the healthcare system. To better understand transmission patterns and evaluate public health responses, we applied phylodynamics, a framework that integrates pathogen genomics with epidemiology. Phylodynamics enables estimation of key epidemiological and evolutionary parameters that help in characterizing disease outbreaks and spread dynamics; and evaluate the interventions including non-pharmaceutical interventions. In this study, we use phylodynamics to analyze time-stamped pathogen genomic data from routine surveillance programs, with the goal of informing actionable public health insights to support decision-making. To achieve this, we collected ~220 influenza A virus hemagglutinin (HA) and neuraminidase (NA) genes and ~500 RSV genomes from the 2023-2024 season. A Bayesian birth-death skyline model and a coalescent skyline model implemented in BEAST2 were used. The median values of time-varying reproduction number (Rt) for H1N1pdm09 is 3.73 (95% HPD: 2.60-5.20) and H3N2 is 3.73 (95%: HPD 1.87-6.45), and the infection duration is 5.11 days (95% HPD: 2.87–7.68) for H1N1pdm09 and 7.51 days (95% HPD: 3.31–13.16) for H3N2. The median of the time to most recent common ancestor (TMRCA) was estimated at 2020.67 (95% HPD: 2020.27–2021.31) for H1N1pdm09 and 2020.80 (95% HPD: 2020.39–2021.16) for H3N2. Interestingly, following the introduction of the vaccine in October 2023, we observed a reduction in the Rt value, indicating a potential impact of the vaccine on transmission dynamics. For RSV, we are currently conducting phylodynamic analysis to understand the co-circulation of both RSV-A and RSV-B genotypes. Overall, these public health use-case scenarios demonstrate the value of phylodynamic modeling in understanding infectious disease dynamics and strengthening genomic surveillance and response efforts.

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NM001 prevents septic-induced myocardial dysfunction by targeting the inflammatory cytokines TNF-α and IL-6

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Background: Patients with severe sepsis can develop acute heart failure characterized by left ventricular dilatation and decreased contractility. Inflammatory mediators, including TNF-alpha, IL-6, and Il-1β, contribute to ventricular remodeling and activating pro-inflammatory pathways in sepsis. However, therapeutic interventions for polymicrobial sepsis are lacking. We hypothesize that administering our RNA-based drug, NM001, would reduce inflammatory cytokine production and improve cardiac function and mortality rate overall.
Methods: To test our hypothesis, we induced polymicrobial sepsis in C57b6 mice via cecal ligation and perforation (CLP) surgery, while mice undergoing sham surgery served as the control group. NM001, an RNA-negative control, or saline were administered IV 6 hours post-CLP. We conducted echocardiographic and hemodynamic assessments at 48 and 168 hrs post-CLP and then sacrificed the mice to collect their hearts for biochemical and molecular analysis.
Results: Administration of NM001 to septic mice 6 hrs post-CLP increased survival, improved echo-derived indices of cardiac function (left ventricular ejection fraction, fractional shortening), increased bacterial clearance, and reduced inflammation when compared to septic mice receiving a scrambled control miR. In addition to TNF-alpha, bioinformatic analysis, confirmed by luciferase assays, identified IL-6 as a specific target of NM001 in cardiac tissue. In septic patients, the expression of the target RNA and their target inflammatory cytokines were decreased in human circulatory blood and in autopsied hearts, which NM001 tries to replace.
Conclusion: Our study's results are promising, demonstrating that NM001 delivery improves outcomes in sepsis-induced myocardial dysfunction. These findings suggest that NM001 could serve as a potential therapeutic target for prevention and prognostic reflection in sepsis-induced myocardial dysfunction, with significant implications for human patients.

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Ecological correlates of the gut resistome following antibiotic treatment

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The gut microbiome is a reservoir for antimicrobial resistance genes (ARGs), termed the resistome. Antibiotics can expand the resistome through selective pressure. Pathogen expansion in the gut has been linked to resistome expansion, suggesting that maintenance of a healthy, diverse gut microbiome may reduce antimicrobial resistance. However, the relative importance of antibiotic regimen and gut microbiome ecology in driving resistome expansion are unclear. We hypothesized that post-antibiotic gut microbiome ecology would be a stronger correlate of the gut resistome than antibiotic class or duration. The BALANCE trial enrolled 3631 participants with bloodstream infections and randomized them 1:1 to receive 7 or 14 days of antibiotics. Antibiotic exposure data was collected every day between enrollment and hospital discharge or death. In 131 participants, rectal swabs were collected within the first seven days of treatment initiation (i.e., enrollment) and days 7, 14, and 21 or discharge. We performed metagenomic sequencing on rectal swabs to taxonomically profile the microbiome and quantify ARGs. Gut microbiome ecological features such as Shannon diversity were stronger correlates of ARG abundance and composition than antibiotic exposure class or duration. These associations remained significant after controlling for antibiotic class and duration. ARG abundance was positively associated with the relative abundance of pathogens, such as Escherichia coli, and negatively associated with commensal anaerobe relative abundance. ARGs primarily originated from pathogens, with E. coli being the largest contributor. To validate these observations, we analyzed publicly available data from an independent cohort of 19 healthy volunteers that were randomized to one of four different five-day courses of antibiotics. Post-treatment ARG abundance was associated with gut microbial ecology, which remained significant after controlling for antibiotic class. Resistome composition was most strongly associated with gut microbial ecology. Minimizing the effects of antibiotics on gut microbiome diversity may be a target to reduce antimicrobial resistance.

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Integrative Prediction and Benchmarking of Protein-Protein Interactions in *Plasmodium falciparum* Gametocytes

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Malaria remains one of the world's deadliest infectious diseases. The WHO's latest World Malaria Report estimates that there were about 263 million cases and 597,000 malaria-related deaths globally in 2023, an increase of 11 million cases from the previous year. Understanding the molecular mechanisms driving parasite transmission is crucial for developing effective interventions. With this goal, we focused on stage-specific protein-protein interactions (PPIs) in Plasmodium falciparum gametocytes, the sexual form of the parasite essential for transmission. Using co-elution mass spectrometry data and protein embeddings from the ESM-2 language model, multimodal deep learning models were developed to predict PPIs in stage 3 and stage 5 gametocytes. True positives were drawn from curated experimental data, and true negatives were generated from co-elution proteins, excluding known interactions. For each stage, five models were trained, validated, and tested using different data splits, and predictions were combined using optimized probability thresholds. The stage 5 model had 2344 predicted interactions amongst 382 proteins, in 84 complexes, and the stage 3 network included 556 interactions amongst 157 proteins in 33 complexes. Network analysis showed that most proteins had a degree of 1, and network hubs included proteins like PfGRP170, an endoplasmic reticulum protein that may contribute to antimalarial drug resistance. Benchmarking using co-expression profiles from RNA-seq data showed significantly higher correlation in expression of predicted PPIs compared to random pairs (Kolmogorov-Smirnov $p < 10\text{-}137$ in stage 5). Ongoing work includes applying phylogenetic profiling to examine co-occurrence patterns across eukaryotic species, detect co-evolved protein pairs, and identify species-specific gains or losses within interaction networks. We also plan to use AlphaFold3 to assess 3D conformations and structural plausibility of predicted interactions. This integrative approach will help prioritize biologically meaningful PPIs and complexes for experimental validation and support the development of stage-specific, transmission-blocking malaria interventions.

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The impact of pre-existing immune activation & coronavirus-specific immune responses on subsequent SARS-CoV-2 incidence & COVID-19 clinical outcomes

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There was a relatively low burden of COVID-19 disease in sub-Saharan Africa (SSA) during the first pandemic wave. We hypothesized that pre-existing immune activation & immune responses to SARS-CoV-2 induced by prior seasonal coronavirus infections would protect against infection and/or symptomatic disease. Female Sex Workers (FSWs) from Nairobi, Kenya had blood collected mid-2019 (pre-pandemic) and approximately 1 year later. SARS-CoV-2 infection was defined by the presence of antibodies against ≥2/3 SARS-CoV-2 antigens. ELISPOT was used to define pre-pandemic T-cell responses against comprehensive peptide pools covering SARS-CoV-2 structural proteins and defined non-structural epitopes. Pre-pandemic serology for the 4 seasonal coronaviruses was also performed using a multiplex immunoassay. Plasma levels of pro-inflammatory markers in pre-pandemic samples were quantified using the MSD V-PLEX proinflammatory panel 1. Immune activation was defined using a composite scoring system composed of the 7 pro-inflammatory markers: IL-1ß, IL-2, IL-6, IL-8, IL-12p70, IFNγ, and TNF. We matched 100 cases who were infected during the first wave with 100 controls, based on age (±5 years) and sample date (±2 weeks). Our primary endpoints and analysis approach were predefined, and all assays were run blind. We found that both the presence and frequency of pre-pandemic IFNy-secreting T-cell responses against structural (but not non-structural) proteins were associated with significant protection against SARS-CoV-2 infection, but not with reduced symptoms among cases. IgG antibodies recognizing seasonal coronaviruses were ubiquitous. There was no significant difference in immune activation score between cases and controls (mean, 17.42 vs. 17.53; p = 0.86); furthermore, levels of the 7 individual cytokines were not significantly different between cases and controls (p>0.05). These results suggest that cross-reactive cellular responses induced by prior seasonal coronavirus infections may protect against SARS-CoV-2 infection but not preceding levels of systemic immune activation.

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Fingerprinting HIV-1 Particles to Discern Cellular Origins

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HIV-1 acquires its lipid envelope during egress from infected cells. In doing so, virions incorporate cellular proteins from the plasma membrane into nascent viral envelopes. Our lab has been extensively characterizing these virion-incorporated host proteins as they can remain functional. For example, our recent work demonstrated the utilization of virion-incorporated CD14 to shuttle bacterial LPS and elicit signaling. Beyond functionality, incorporated proteins also offer researchers a tool to discern virus origins. We hypothesize that HIV-1 from different tissues may incorporate proteins that reflect the unique producer cell types, thereby allowing the inference of the cellular source of HIV-1 reservoirs.

Herein, we propagated HIV-1 in donor-matched peripheral-blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs). Virus-containing supernatants were then analyzed by mass spectrometry. We discovered that, while MDM- and PBMC-derived HIV-1 share a large fraction of identified proteins, there were significant differences in the enrichment of those proteins between the two groups. We also demonstrate that PBMC-derived viruses more closely relate to PBMC cell lysates than do MDM-derived viruses. Deconvolution attempts also highlight cells types that contributed to the phenotypes or reflect the manner of presentation to immune cells. We next aim to validate the detected proteins by flow virometry and perform similar analyses on clinical samples with the goal of identifying key contributing cell types to circulating virions in patients with rebound viremia.

A key challenge in HIV cure strategies is identifying the viral reservoirs to target and eliminate systemic shedding. The goal of this work is to determine how characterizing the human proteins on virions might improve our understanding of HIV-1 reservoirs. Our results highlight the ability to discern PBMC- and MDM-derived HIV-1 based solely on these virion-incorporated human proteins, reinforcing our hypothesis that virions emerge from infected cells with a unique protein fingerprint that can inform cellular origin.

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Multi-colour Flow Virometry to Understand Protein Profiles of HIV

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The Human Immunodeficiency Virus (HIV) can incorporate a large arsenal of human proteins through budding from an infected cell. Our research demonstrates many of these newly acquired proteins can influence viral-homing, attachment, and inflammatory responses. Despite its low transmission efficiency, HIV's ability to modulate its surface protein composition could offer significant adaptive advantages, aiding its persistence and spread throughout the body. Given that just one virus, among a myriad of diverse particles, is required to establish an initial infection, the contributions of individual virion phenotypes become crucial in understanding infection and designing effective therapeutics. Our lab has been establishing new methods in Flow Biometry (FV) for high-throughput quantification of surface proteins on individual virions. In this current proof of principle study for assay development we developed techniques for multi-colour FV to detect the co-incorporation of multiple antigens on the same particles. First, we employed HIV pseudoviruses to simultaneously stain multiple proteins on individual particles. We then applied these techniques to three distinct HIV isolates propagated in PBMCs or T-cell lines, observing natural patterns of co-incorporation. Finally, in order to better understand how these individual virion phenotypes may affect HIV biology, one must isolate uniform virus subpopulations for controlled studies, which has been exceptionally challenging to achieve with conventional techniques. Therefore, as our third set of experiments, we created a heterogenous mix of HIV pseudoviruses and demonstrated that they can be sorted into homogenous populations with a new generation of benchtop nanoparticle sorter. This study showcases the development of a new tool for studying viral heterogeneity. In future, we hope to apply this multi-colour FV to study virus subpopulations circulating in infected patients in efforts to discern their contributions to disease progression and viral spread.

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Looking Beneath the Viral Envelope using Flow Virometry

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Introduction: Virus preparations are universally contaminated with extracellular vesicles (EVs) that share the size and surface protein composition of enveloped viruses like HIV. Differentiating viruses from EVs using flow virometry that analyzes nanoparticles based on the same two parameters, thus becomes challenging. Alternatively, using flow virometry to detect the structural proteins most abundantly present inside viruses than EVs may enable us to overcome this challenge. Drawing from the well-established technique of intracellular staining in flow cytometry, here, we have performed intravirion staining targeting the abundant capsid protein of HIV-1, known to be minimally incorporated in EVs, and analysed the samples using flow virometry.

Methods: Herein, we first stained HIV-1 pseudoviruses with 2 different anti-p24 antibodies, namely, KC57 conjugated to R-phycoerythrin, and 28B7 conjugated to APC. The viruses were permeabilized using different permeabilizing agents and techniques. Unpermeabilized viruses were used as controls. After identifying the best permeabilizing agent and protocol, we stained these viruses with KC57-FITC antibody. We also performed a simultaneous surface and intravirion staining of these pseudovirus models expressing a known human protein. Additionally, we also performed intravirion staining of primary cell derived HIV-1 using KC57-FITC antibody.

Results and Conclusions: Our results show that intravirion staining can be performed by selecting the correct permeabilizing agent, experimental protocols, antibody clones and the conjugated fluorophore. Moreover, our results demonstrate that the surface staining of viruses can be retained and detected simultaneously with intravirion staining on the same particles. Intravirion staining using flow virometry can have a wide range of applications including accurate identification of viruses, identification of intraviral proteins, genetic material or lipids, novel intraviral host proteins incorporated in viruses and antiviral compounds aiding in downstream biological assays. These techniques can also be applied to EV research to identify novel intravesicular cargo and their roles in physiologically relevant context.

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Neisseria gonorrhoeae infection in the genital tract alters the murine gut microbiome

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The ability of Neisseria gonorrhoeae (Ngo) to infect and persist in the genital tract has previously been shown to be modulated by resident microbiota. The Lactobacillus species that often dominate these communities have been associated with protective effects in in vitro experiments against Ngo and other STIs, and their presence is enriched within asymptomatic presentations of gonorrhea. The vaginal microbiome is affected by multiple factors including diet, sexual activity and, notably microbial communities associated with the gastro-intestinal tract. Of particular interest is the bidirectional relationship between gut and vaginal microbiomes, their impact on health, and the microbial, metabolic, and immune factors driving their interactions. To better understand the impact of vaginal gonorrheal infection on murine vaginal and gut microbial communities, we exploited a mouse model for vaginal N. gonorrhoeae infection. We performed 16S rRNA and whole microbiome DNASeq (metagenomics sequencing) to profile community composition and function respectively. Our murine model demonstrated Ngo established a strong but transient infection in the vaginal environment, responsible for >66% of 16S bacterial sequencing reads one day post infection (dpi). Surprisingly, beta diversity differences were detected only in the gut, where one dpi infected samples clustered significantly differently from uninfected samples (PERMANOVA p = 0.012). Further correlational analyses revealed a cluster of 9 gut families, including Lachnospiraceae and Ruminococcaceae, that correlate positively with Ngo. From a functional perspective our metagenomics data reveals that differentially abundant genes within infected vaginal communities are enriched for purine nucleotide binding and adenyl nucleotide binding terms . Vaginal infection of N. gonorrhoeae corresponds to changes in prominent gut families and beta diversity. Functional changes in the vaginal microbiome may be linked with host purinergic signalling or nucleotide metabolism. A second study, to explicate upon these results with larger sample size and sera collection, has been completed.

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Ventilator Associated Pneumonia – Prevalence and Microbiology of an Acute Care Hospital

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Background
Ventilator-associated pneumonia (VAP) is a healthcare associated infection in Intensive care unit (ICU) patients on mechanical ventilation, associated with increased morbidity, mortality, and costs. The rise in antimicrobial resistance is a growing concern, with more resistant pathogens identified globally. Despite advances in diagnosis and treatment, VAP remains a significant ICU challenge. This study examines the prevalence, causative agents, and antimicrobial resistance trends in VAP cases at our hospital.
Methodology
This 3-year retrospective study in the ICUs of an acute care hospital included patients diagnosed with VAP based on Ontario's Critical Care Services criteria, in consultation with an infectious disease physician or intensivist. The VAP rate was calculated as confirmed VAP/ventilator days × 1000. All respiratory culture samples were processed at the Shared Hospital Laboratory where causative agents and antimicrobial sensitivity were identified.
Results
VAP rates for April 2022-March 2023, April 2023-March 2024, and April 2024-March 2025 were 1.81, 3.54, and 3.90 per 1000 ventilator days, respectively. The most common pathogens were Pseudomonas aeruginosa (25%), Staphylococcus aureus (19%), Stenotrophomonas maltophilia (9%), and Methicillin Resistant Staphylococcus aureus (MRSA) (9%). In addition to the mentioned, other Gram-negative bacteria (Enterobacter cloacae complex, Proteus mirabilis etc.), Gram positive bacteria (Staphylococcus aureus, Streptococcus pneumoniae) and yeast were isolated.
A rise in antimicrobial resistance among VAP pathogens was noted in 2024, with first-time isolation of Carbapenemase-producing Enterobacteriaceae (CPE), multidrug-resistant Acinetobacter baumannii and an increase in MRSA. Resistance in Pseudomonas aeruginosa to Ceftazidime (0% in 2022 to 59% in 2024) and Piperacillin-Tazobactam (0% in 2022-2023 to 25% in 2024) also increased.
Conclusions
VAP can be caused by various pathogens with resistance mechanisms, complicating treatment. In the era of widespread antimicrobial resistance (AMR), understanding VAP prevalence, adherence to VAP bundle, early diagnosis, prompt treatment, adherence to antimicrobial stewardship, and robust infection control practices can help reduce VAP cases.

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A *C. elegans* Stress Response to *Actinobacteria*

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Stress responses are universal in organisms and have evolved for survival against a broad range of threats. When looking at Caenorhabditis elegans, which are soil-dwelling, bacteria-eating nematodes, we see that a P-Glycoprotein (PGP), a putative drug exporter, is upregulated when exposed to Cationic Amphiphilic Drugs (CADs). CADs induce phospholipidosis, a lysosomal storage disorder that can result in cellular and tissue dysfunction. The upregulation of the PGP implies that CADs are toxic compounds that C. elegans need to protect against through a CAD-defense system. However, known CADs are not used to kill worms and instead are usually synthetic drugs used to treat various human ailments. This raises the question, “Why do C. elegans have a CAD-defense system?”. To answer this, I investigated the natural environment of C. elegans. Soil is abundant with Actinobacteria, which are known to secrete various specialized metabolites that can kill nearby organisms to protect themselves from predators and gain competitive ecological advantages. I hypothesize that Actinobacteria are responsible for driving the evolution of the CAD-defense system in C. elegans, where Actinobacteria secrete toxic CADs into the soil, resulting in C. elegans without a defense system to die. With an in vivo transcriptional fluorescent reporter, I found that few Actinobacteria induce PGP expression, implying CAD production. Sequencing and mass spectrometry will elucidate the compounds responsible for triggering the CAD-defense system. This will identify novel natural product CADs or provide ecological contexts to known specialized metabolites. Studying the mechanism and metabolites involved with the CAD-defense system in C. elegans will deepen our insights on CAD-induced phospholipidosis, possibly aiding the efforts in mitigating symptoms. Additionally, examining Actinobacteria and their effect on C. elegans genetics will aid our understanding of how stress response evolution occurs, furthering our knowledge of the emerging issue of anthelmintic resistance.

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Understanding cell type specific effects of bacterial infection in primary Cystic Fibrosis epithelium

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Cystic Fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) chloride ion channel. The major mutation, F508del, results in a loss of functional CFTR at the plasma membrane, disrupting CFTR-mediated fluid secretion and absorption in airway epithelial cells. This results in a less fluid mucosal layer which decreases the host ability to clear pathogens and promotes chronic infection. The most commonly isolated bacterium from CF patients is the Gram-negative opportunistic pathogen, Pseudomonas aeruginosa, which causes chronic inflammation and tissue damage. Our goal is to study the host response to P. aeruginosa in CF and healthy tissue.

I will use primary human airway epithelial cultures as a model system. Previously obtained single-cell RNA sequencing (scRNAseq) data from these cultures revealed a variety of different cell types. Moreover, single-cell data comparing CF and healthy cultures revealed that CF patients have differential cell type abundance. This led us to hypothesize that there is a cell type specific response to pathogens in the airway epithelium, which is altered as a result of the altered cell type composition within CF tissues.

To test this idea, I will utilize a live-cell microscopy based calcium flux assay to monitor cell type specific responses to exudates isolated from P. aeruginosa. I can also use cell type specific markers derived from scRNAseq results to validate calcium responses originating from distinct cell types.

Overall, elucidating the cell type specific effects to bacterial stimuli will be crucial in understanding the proinflammatory status of CF tissue and will help explain the altered abundance of distinct cell types in CF patients. Results will define appropriate cell type specific receptors as therapeutic targets to help reduce inflammation in CF patients and improve their quality of life.

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Splicing HIV to Sleep: SR Kinase Inhibitors as a Strategy for Achieving a Functional Cure for HIV

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HIV-1 replication depends on interactions between the virus and host factors, particularly in RNA processing. The virus exploits host alternative splicing to generate over 100 alternative spliced viral mRNAs. This delicate orchestration of these transcripts is pivotal, allowing balanced expression of all viral proteins necessary for 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 HIV-1 replication. SR (Serine-Arginine) proteins are conserved host proteins involved in both host and viral RNA processing. We curated a chemical library of SR kinase inhibitors and identified several compounds able to inhibit HIV-1 replication. We present evidence on the capacity of select compounds to reduce viral RNA and protein accumulation, impact on RNA synthesis, stability, or changes in alternative splicing site usage. We observed that despite a similar effect on HIV-1 gene expression, compounds had distinct effects on SR protein phosphorylation and viral RNA splicing that are correlated with altered expression of distinct SR kinases, previously shown to affect viral replication. 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 HIV.

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A role for SR proteins in the maintenance of HIV-1 latency

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The major barrier to a cure for HIV-1 is the reservoir of latently infected cells, consisting primarily of resting memory CD4+ T cells. Recent studies have highlighted issues in viral RNA processing as one component of the latency barrier. Consequently, understanding how HIV-1 transcription and RNA splicing regulation contributes to latency is critical for developing curative therapies for HIV-1. Key to regulating RNA processing are the host SR (serine-arginine rich splicing factors) proteins. As part of our effort to understand the role that individual SR proteins play in regulating HIV-1 replication, we examined their interaction with HIV-1 RNA and the effect of their depletion on viral gene expression. eCLIP analysis determined that these factors had extensive overlap in their binding sites on the viral RNA. Despite similar binding profiles, of the eight SR proteins (SRSF1-7, 9) tested, depletion of only SRSF3, 5, and 9 yielded marked increases in HIV-1 protein expression and viral RNA accumulation in two T cell lines examined (JLat 10.6, CEM-T4). Unexpectedly, the regulation of HIV-1 expression by SRSF3 and SRSF5 predominantly occurs through their impact on HIV-1 RNA transcription initiation, as evidenced by pulse-labelling and luciferase assays. While SRSF3 & 5 depletion increased HIV-1 promoter function, loss of SRSF9 affected post-initiation events which could be elongation, splicing or polyadenylation. In the context of CEM-T4 cells, increased HIV-1 gene expression was predominately due to a >14-20 fold increase in the percentage of cells expressing Gag upon SRSF3 or SRSF9 depletion, an effect that could be further augmented by addition of various latency reversing agents. Effects on promoter function were not unique to HIV-1 as subsequent analyses determined that SRSF3 or 9 depletion also modulated promoter function of multiple host genes. Together, these observations highlight an unexpected role for SRSF3, 5, and 9 in the regulation of HIV-1 latency through affects on viral promoter function.

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A 16-Year-Old with a *Mycoplasma hominis* Empyema Post-Lung Transplantation: A Case Report

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BACKGROUND: This is the first reported case of empyema due to Mycoplasma hominis in a pediatric transplant recipient.
METHODS: A 16-year-old Indigenous Canadian boy developed acute respiratory distress 29 days post bilateral lung transplantation for chronic lung disease and pulmonary hypertension secondary to extreme prematurity and an atrial septal defect. Pre-transplant donor bronchial cultures grew Candida albicans and methicillin-sensitive Staphylococcus aureus, so he received 14 days of cefazolin. Post transplant prophylaxis included azithromycin, voriconazole, micafungin, TMP-SMX and valacyclovir. Immunosuppression included anti-thymocyte globulin induction followed by tacrolimus, mycophenolate mofetil and prednisone.
The patient developed a large right pleural effusion over the course of 24 hours requiring ICU transfer and high-flow supplemental oxygen. Pleural thoracentesis revealed a neutrophil predominant exudative empyema. Routine cultures were negative; M. hominis was detected by PCR and specialized media. The patient completed 28 days of clindamycin and doxycycline and made an uneventful recovery.
RESULTS: M. hominis and Ureaplasma species are donor-derived pathogens that can cause significant morbidity, including sternal wound infection, mediastinitis, pericarditis and empyema. Post-lung transplant M. hominis infections occur in 2-5% of cases. Diagnostic challenges, low clinical suspicion and rising resistance contribute to poor outcomes and inappropriate antibiotic use. While this patient's ammonia level was normal, hyperammonemia syndrome also remains a rare but serious complication of Ureaplasma urealyticum and M. hominis infections.
CONCLUSION: Early screening, PCR testing and prompt empiric therapy are crucial for improving outcomes in M. hominis infections.

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Genomic characterization of non-invasive isolates of *Streptococcus pyogenes* collected in the Greater Toronto Area from 2023-2025

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Background: As a major cause of morbidity and mortality worldwide, Streptococcus pyogenes (Group A Streptococcus, GAS) is responsible for approximately 700 million infections annually. These include both invasive (e.g., bacteremia) and non-invasive infections (e.g., pharyngitis). Little is known about the circulating non-invasive GAS isolates. Here we investigated the genomic epidemiology of circulating non-invasive isolates over the 2023-2025 respiratory virus seasons.

Methods: Non-invasive isolates were collected from individuals who presented at 10 hospitals in the Greater Toronto Area between February 2023 and March 2025. Invasive isolates were collected between November 2023 and December 2024. DNA was extracted using the easyMag system (BioMérieux), and libraries were prepared using the Nextera DNA Prep kit (Illumina). Sequencing was done with paired-end reads on the MiniSeq platform.
Sequencing data was analyzed using the Bactopia pipeline (v3.0.1) with default settings. Emm-typing was done via the Bactopia emm-typer workflow (v0.2.0). A maximum-likelihood phylogenetic tree was inferred via the Bactopia pangenome workflow using Panaroo (v1.5.2). The tree was visualized using Microreact.

Results: 996 isolates were sequenced to date (543 invasive and 453 non-invasive). Phylogenetic analysis revealed multiple distinct clusters, indicating high genetic variation among circulating isolates. Among all the isolates, 49 emm-types were identified, with the most prevalent subtypes being emm1 (271 isolates) and emm12 (161 isolates). Specifically, emm1, emm92, and emm74 were the most common among invasive isolates, whereas emm12 and emm1 were the most prevalent among non-invasive isolates. Additionally, emm49, previously associated mainly with invasive isolates, was also found in non-invasive isolates during 2023–2024.
Preliminary analysis of virulence factors did not indicate differences in prevalence among the most commonly identified emm-types (emm1and emm12). No strong clustering pattern was observed between emm-types and hospital sources, suggesting wider community transmission.

Conclusion: The high emm-type diversity and geographical variability in circulating strains highlight the importance of GAS surveillance and phylogenetic analysis.

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Pediatric invasive pneumococcal disease (IPD) in Ontario late after the introduction of a routine infant PCV13 program

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Introduction/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 and analysis: From 2014-2023, 443 pediatric (age<18y) IPD cases occurred, with clinical data available for 413 (93%), serotyping for 426 (96%). 47 (11%) cases required ICU, 28 (7%) had meningitis, 9 (2%) died. 310/443 (70%) were <5y; 156/413 (38%) had an underlying condition predisposing to IPD (with 82, 20% immunocompromised). Underlying conditions were more common in 5-17y-olds (53% any, 37% immunocompromised). 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, 105/426 isolates (24.6%) were PCV13 STs, 56 (13.2%) PCV15/notPCV13, 122 (28.6%) PCV20/notPCV15, 143(33.6%) were non-PCV. PCV13 ST were: 49 ST 19A (47%), 39 (37%) ST 3, 11 (10%) 19F, 3(3%) 7F, 1 each 14, 9V, 23F. Of 92 (88%) with evaluable vaccine history, 29 were not eligible (5 <2mos, 24 too old to have received PCV13), 40 were vaccine failures, 18 were program failures (10 unvaccinated, 8 no >12m dose); 5 incompletely vaccinated.

Conclusions and implications for policy, practice or additional research: 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.

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Incidence and clinical characteristics of invasive group A streptococcal infections in underhoused adults in Toronto, Canada

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Background: Post-pandemic increases of invasive group A streptococcal disease (iGAS) have been reported in many areas of the world; increased risk associated with illicit intravenous (IV) drug use has also been identified.
Methods: Since 1995, the Toronto Invasive Bacterial Diseases Network (TIBDN) has conducted prospective, population-based surveillance for iGAS in residents of Toronto/Peel Region (pop, 4.5M) in Canada. Homeless adults admitted to hospitals in the surveillance area are counted as residents. General and homeless population estimates were taken from Statistics Canada and published literature (doi:10.1136/bmjopen-2019-030221). Canada's National Microbiology Laboratory performs emm typing.
Results: In 2022/23, 88 iGAS cases (14% of all iGAS, 17% of adult iGAS) occurred in homeless persons; all were adults. Estimated iGAS incidence in homeless adults was 210/100,000/yr, versus 5.4/100,000/yr in other adults. Homeless persons with iGAS were younger than other adults (median age 47 vs 59 yr, P<.001); 70% were male (v 63% in other adults, P=0.2. Homeless adults were more likely than other adults to be smokers (53% v 27% P<.001), to use IV drugs (46% v 10%, P<.001), to have non-intact skin (43% v 27%, P<.001) and to report recent soft tissue trauma (27% v 17%,P.<.001). Compared to other adults, sixteen (18%) homeless adults required ICU admission, 10 (11%) mechanical ventilation, and 5 (5.8%) died. After age adjustment, outcomes did not differ between homeless and other adults. The median hospital length of stay was 8.5 days (IQR 4-22) for homeless adults, and 8 days (IQR 5-18) for other adults (P=0.7). The distribution of emm types of isolates in homeless and other adults is shown in Figure 1. iGAS in homeless adults was less likely to be caused by isolates of emm 1 and 12, and more likely to be caused by isolates of emm49 and 74.
Conclusions: Homeless persons are at very high risk of iGAS, with IV drug use and skin integrity issues contributing. Outcomes of iGAS as are similar to other adults. Differences in the emm types of isolates may be relevant to future vaccine program planning.

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Should pneumococcal conjugate vaccines be recommended for houseless adults? Evidence from population-based surveillance for Invasive pneumococcal disease in Toronto/Peel region.

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Introduction/background: Pneumococcal conjugate vaccines (PCVs) are recommended for immunocompromised and all older adults; whether PCVs should be recommended for other adult populations remains uncertain. We assessed the epidemiology of invasive pneumococcal disease (IPD) in houseless persons in Toronto/Peel region from 2014-2023.

Methods: TIBDN performs population-based surveillance for IPD in Toronto/Peel region (pop 4.5M). Houseless adults presenting to hospitals within the population area are considered residents. Microbiology laboratories serving area residents report sterile site isolates of S. pneumoniae; annual audits ensure completeness. Isolates are serotyped at Canada's National Microbiology Laboratory. Population data estimates are from Statistics Canada and published literature (doi:10.1136/bmjopen-2019-030221e030221).

Results and analysis: Of 2182 adult IPD cases from 2014-2023, 231 (10.5%) occurred in persons who were houseless (226) or reported being houseless (5) in the last year. All IPD cases in houseless persons were in in adults; 38 (16%) were ≥65 years; 48 (21%) were female. In 2022/23, the estimated incidence rate of IPD in houseless adults was 149/100000/year, compared to rates of 2.9 and 15.2/100000/year for other adults aged 18-64 and ≥65yrs, respectively. Compared to other adults, houseless persons with IPD were more likely to have any underlying illness (83% vs 66%), to abuse alcohol (44% vs 11%), to smoke (80% vs 28%) and to use IV drugs (21% vs 3%), but less likely to be immunocompromised (15% vs 34%) (all P<.001). Overall, 37% of houseless persons required ICU admission, and 13% died; median hospital length of stay was 6 days (IQR 3-14). In adjusted models, outcomes were not different in houseless and other adults. Isolates of serotypes included in PCV20 and V116 caused 69% and 62% of IPD in houseless persons, as compared to 61% and 77% of IPD in other adults.

Conclusions and implications for policy, practice or additional research: In our population, houseless persons are at strikingly high risk of IPD and should be included in recommendations for publicly funded vaccine programs.

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A South American Mouse Morbillivirus Provides Insight into a Clade of Rodent-Borne Morbilliviruses

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Morbilliviruses are negative-sense, single-stranded, monosegmented RNA viruses belonging to the family Paramyxoviridae (order Mononegavirales). These viruses infect a wide range of mammalian hosts, including humans, dogs, cats, small ruminants, seals, and cetaceans. Here, I report the identification and characterization of novel morbilliviruses discovered through the mining of publicly available RNA-seq datasets from South American long-haired and olive field mice. These divergent viruses, termed Ratón oliváceo morbillivirus (RoMV), were detected in renal tissue samples from mice collected in Chile and Argentina. RoMV exhibits an unusually large genome, featuring extended intergenic regions and an accessory protein encoded between the F and H genes, resulting in the unique genome architecture: 3′-N-P/V/C-M-F-hp-H-L-5′. Comprehensive structural and functional annotation, alongside genetic distance metrics and phylogenetic analyses, support the classification of RoMV as a member of a novel species within the genus Morbillivirus. Notably, RoMV clusters within a monophyletic clade of newly described rodent-associated morbilliviruses, collectively forming a distinct sublineage. This group expands the known host range of morbilliviruses, reshapes our understanding of their genomic architecture, and provides novel insights into their evolutionary trajectories. Recently, RoMV has been formally recognized by the ICTV as part of a novel species within the newly established genus Paramorbillivirus, which currently includes four rodent-borne viruses. Given the high mutation rates and host adaptability of morbilliviruses, the discovery of rodent-borne lineages underscores a potentially underrecognized zoonotic risk, with significant implications for infectious disease surveillance and global health preparedness.

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Interactions with phage are a major driver for *Legionella pneumophila* antigenic diversity and virulence

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Legionella pneumophila is the causative agent of a severe type of pneumonia called Legionnaires' Disease. The bacteria generally cannot spread from person-to-person, so humans are considered an evolutionary dead-end, hence the adaptations that make L. pneumophila pathogenic are coincidental to their fitness in the natural environment. Co-evolution with bacteriophage is one common driver of bacterial natural selection. Recently, our lab validated the first infectious L. pneumophila bacteriophage: Legionella Mobile Element-1 (LME-1). Very little is known about a Legionella-phage dynamic, but through early studies of this novel phage-host pair, our lab has established a link between a well-characterized virulence gene, lag-1, and LME-1 resistance. Lag-1 protects L. pneumophila from LME-1 attachment likely by obstructing access to the phage receptor. A phage's strategy to engage with receptors on the bacteria's surface to initiate an infection informs its host range. Based on the characteristics of its outer membrane, L. pneumophila can be divided into 15 serogroups. I have shown that LME-1 attachment is restricted to two distinct serogroups, specific subgroups, and in some cases, a specific growth phase. Using comparative genomics, I have identified a candidate gene to explain serogroup specificity. Phages contribute to antigenic diversity within a bacterial species as the host frequently modify their surface receptors to protect themselves from phage adsorption. Often, in the case of pathogens, these modifications can have an effect on their virulence. The link between lag-1 and LME-1 resistance reveals a previously unknown environmental role for the gene. This suggests that other distinct L. pneumophila surface features with unknown functions may have arisen as alternative methods to resist phage adsorption. This work will shed light on the origin of L. pneumophila's distinct surface characteristics related to LME-1 susceptibility and their contribution to environmental fitness and human virulence.

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Digoxin as a host-directed antiviral against human adenovirus

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Human adenovirus (HAdV) infections are often mild, but can be serious (e.g., pneumonia, epidemic keratoconjunctivitis, hepatitis) in both immunocompromised and healthy individuals. In pediatric bone marrow transplant recipients, disseminated HAdV infections have a fatality rate greater than 50% (Lion T, 2014, Clin Microbiol Rev 27(3):441-462). There are currently no approved antivirals specific for HAdV. Our lab has shown that digoxin, a drug for heart failure, suppresses replication of HAdV species A-D in cell culture, as determined 24 hours post-infection (p.i.) (Grosso et al., 2017, J Virol 91(3):e01623-16). The current study revealed that virus replication does occur in the presence of digoxin, but it is delayed and overall levels of progeny virus are reduced about 100-fold. Specifically, digoxin delays transcription of E2B (which encodes DNA polymerase and pre-terminal protein), genome replication and late transcription, and further delays progeny virus accumulation, suggesting that digoxin may also affect processes beyond viral genome replication and late transcription. Digoxin not only affects virus replication, but it also compromises host cell replication. Extended incubation of cells with digoxin led to isolation of a clone (A549dig) that requires a 25-fold higher concentration than parental cells to inhibit both virus and cellular growth. Digoxin binds to the sodium-potassium ATPase (NKA) on the cell surface, altering the intracellular ionic balance and affecting multiple signaling pathways (Ren et al., 2024, Bioorg Med Chem 15:114:117939). We showed that A549dig cells have a mutation affecting the binding pocket of the NKA, possibly reducing the binding of digoxin and thereby the cells' increased tolerance to digoxin. This work is relevant to studies targeting host cell processes as a strategy for development of antiviral agents.

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CRISPR-based Diagnostics for the Detection of *Neisseria gonorrhoea* and Antimicrobial Resistance

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The World Health Organization (WHO) and the Foundation for Innovative New Diagnostics (FIND) have emphasized the critical need to expand screening for sexually transmitted infections (STIs), particularly in low- and middle-income countries (LMICs). Gonorrhoea, a bacterial STI of particular concern, affects approximately 87 million individuals annually. Infections can often lead to severe reproductive health consequences, particularly in women. If left untreated, complications include infertility, pelvic inflammatory disease, and increased susceptibility to HIV. Despite the significant public health burden, diagnostic tools in LMICs remain limited by cost, complexity, and resource requirements. In response, FIND has released target product profiles to guide new diagnostic development. One is for a rapid, affordable gonorrhoea test, and another to assess gonorrhoea's antimicrobial susceptibility, addressing the rise in antimicrobial resistance (AMR) as strains increasingly evade available antibiotics.

Current diagnostic methods, such as nucleic acid amplification tests (NAATs), are effective for detecting gonorrhoea but are expensive and dependent on laboratory infrastructure, limiting their utility in LMICs. To bridge this gap, our project focuses on developing a CRISPR-based biosensor for detecting Neisseria gonorrhoeae (NG) and AMR-associated variants. The CRISPR-Cas system leverages guide RNA and CRISPR-associated (Cas) enzymes to target conserved DNA sequences in NG and mutations linked to AMR. Detection is then enabled by the nuclease activity of Cas enzymes, which cleave reporter molecules upon recognizing the target sequence

Our diagnostic platform will be optimized for isothermal, power-free detection, making it ideal for limited-resource settings. Results will be displayed via a low-cost lateral flow assay, optimized for sensitivity, specificity, and rapid time-to-result. Our assay aims to deliver a diagnostic tool that supports cost-effective STI screening, improves AMR surveillance, and reduces transmission, paving the way for better health outcomes in LMICs.

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Validation of the BD Kiestra MRSA Application for detection of MRSA from Surveillance Specimens on BD BBL CHROMagar MRSA II

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Objectives
The BD Kiestra™ MRSA Application (MRSAApp) uses high-resolution plate images captured by the BD Kiestra™ Total Laboratory Automation (TLA) system for automated digital segregation of cultures planted on BD BBL™ CHROMagar® MRSA II plates (CA). Results generated by the MRSAApp are either growth of mauve colonies suggestive of MRSA or MRSA negative indicating no growth or absence of mauve colonies. This study evaluates the MRSAApp's accuracy in its result classification.
Method
Anterior nares or rectal swabs collected with a single ESwab (Copan) and nares/rectal swabs collected with a dual ESwab submitted for MRSA surveillance were included. Swabs were processed by TLA to CA and incubated at 37°C. Plates were imaged and analyzed by both the MRSAApp and medical laboratory technologists (MLT). Mauve colonies suggestive of MRSA were processed for confirmation using standard procedures by an MLT. The MRSAApp's segregration were retrospectively compared to final culture results to assess accuracy.
Results
Of 2524 consecutive MRSA surveillance swabs, 46 were confirmed MRSA-positive (1.8% positivity rate). The MRSAApp correctly classified 45/46 (97.8%) MRSA-positive swabs as potential MRSA, with one false-negative due to a single mauve colony. Additionally, 168 specimens were classified as potential MRSA but did not yield MRSA, plates demonstrated lighter mauve colonies and/or agar discoloration at the inoculation site. Overall, the positive predictive value (PPV) of the MRSAApp was 21.1%, and the negative predictive value (NPV) was 99.96%.
Conclusion
MRSAApp is an effective tool for automated screening of MRSA plates. Although PPV was low, the high NPV would lead to a significant reduction of manual workload. With approximately 75,000 MRSA processed annually, MRSAApp could automatically segregate 92% of specimens as negative, with only 8% requiring manual MLT review. Enabling autoverification of negative cultures can further streamline workflow and optimize MLT time to prioritize work-up of mauve colonies suggestive of MRSA.

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Investigating the effect of quorum sensing on anti-phage defences in bacteria

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The rise of bacterial infections has caused pressure to develop novel therapeutics, such as using bacteriophages (phages). Phages are viruses that infect, replicate within, and kill bacteria. Unlike antibiotics, they can precisely target different pathogens with less microbiome disruption. To harness and engineer phages effectively, we need to understand how bacteria resist infection, as they encode anti-phage defense systems that provide multi-layered resistance. Some defenses 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. This is thought to be evolutionarily advantageous as production of anti-phage defenses 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 Shango and Gabija anti-phage systems, which had not yet shown activity in the native context. 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 defense 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 both Gabija and Shango, and future investigation will determine whether the network drives expression of other systems.
This work will reveal important information about the regulation of multiple anti-phage systems in the important human pathogen P. aeruginosa. We will gain insight into how bacteria present a multi-layered defense and the triggers that induce the expression of these systems. In the long term, this knowledge will contribute to developing effective phage therapeutics that can evade these defenses.

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Communication, Community, and Covid-19

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Background: Covid-19 Resources Canada is a volunteer-based organization that provides knowledge outreach to Canadians about Covid-19. We assessed the needs surrounding their new Journal Club meeting to understand how best to structure science communication for a digital community of individuals continuing social distancing and masking measures. Methods: We used qualitative methods, including thematic coding using NVivo to extract themes from records of a prior meeting as well as from a focus group we conducted. We used quality-improvement frameworks to conduct needs analysis. Results: We found that although practical elements of science literacy were important, audience members felt a desire to be able to communicate science and health information within their communities. They found that successful instances of communicating health needs and concerns to receptive peers were validating and had a positive impact on their sense of safety and inclusion in the community. Unsuccessful communication about their health concerns, particularly in medical settings, led to considerable frustration and anxiety, as well as feelings of "betrayal". Conclusions: Science communication around stigmatized or contentious health topics should account for the role of peer-to-peer and patient-to-provider communication in psychological and social stresses.

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Who is at high risk of influenza complications? An international comparison of population eligibility for annual influenza vaccination

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Influenza is a common respiratory infection with a high global burden.(1) Annual vaccination is the best prevention method.(1) While there is general agreement that vaccination is especially important for people at high risk of influenza complications, interpretation of the evidence varies by country.(1) We conducted a search of government websites of 42 high-income countries to identify adult populations for whom annual influenza vaccination is recommended. Nine countries (21%) recommend vaccination for all adults (18+ years); 31 countries (74%) recommend vaccination for all adults over 50 (n=2), 55 (n=2), 60 (n=7), and 65 (n=20) years of age; while two countries (5%) recommend vaccination only for adults with comorbid conditions placing them at particular risk of complications.

Forty countries (95%) define at-risk health conditions as a part of influenza vaccine recommendations. Thirty-nine include chronic respiratory and immunocompromising conditions. Cardiovascular conditions are included in 37 countries, with heart failure (n=10) most commonly specified. Six countries exclude and 3 specifically include hypertension in cardiovascular risk. Diabetes, chronic renal, and liver conditions are included in 35, 36 and 28 countries, respectively. Elevated BMI is included by 21 countries, defined as over 40 (n=15), 35 (n=1), 30 (n=4), and 25 (n=1). Neuromuscular conditions causing risk of aspiration are mentioned by 11 countries. Thirty-two countries use a comorbidity criterion for vaccine recommendations (2 for all adults; 29 for all adults younger than those meeting their age criterion; and 1 for adults aged 60-65).

The substantial global variation in the definition of populations at sufficient risk of influenza to warrant annual vaccination highlights the need to understand the reasons for variability to ensure equitable and evidence-based protection against influenza for all.

1. Organization WH. Influenza (seasonal): World Health Organization; 2025 [updated February 28th, 2025. Available from: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal).

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Pre- and Post-Pandemic Incidence of Carbapenemase-producing Enterobacterales (CPE) in Toronto and Peel Region, Ontario.

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Background: CPE are an increasing public health threat globally. We assessed the epidemiology of CPE in Toronto and Peel Region during and after the COVID-19 pandemic.

Methods: TIBDN has performed population-based surveillance for CPE since first detection in Oct 2007. All hospitals serving residents and community laboratories serving >80% of residents report isolates to a central laboratory. Population data are from Statistics Canada.

Results: From 2007-2024, 2250 persons were identified as colonized/infected with CPE. Males comprise 56%, median age is 69 years. 1005 (45%) persons had ≥1 clinical isolate: 164 (16%) were bacteremic. The annual incidence of cases with CPE clinical isolates increased from 0 in 2006 to 3.99 per 100,000 population in 2024. The steady increase was interrupted briefly by the COVID-19 pandemic, with a 24% decrease from 2019 to 2020-21 (IRR 0.73, 95% CI 0.55-0.97) but a steeper increase since (compared to 2019, IRR for 2024 was 2.14, 95% CI 1.66, 2.78). The most frequent species were E. coli (44%) and K. pneumoniae (33%). The most common carbapenemases were NDM ± OXA (53%), OXA (26%), and KPC (16%). NDM and OXA have been the major contributors to the increased CPE incidence since the pandemic (NDM±OXA: 2024 vs 2019, IRR 2.59, 95%CI 1.82-3.75; OXA: 2024 vs 2019, IRR 1.99, 95%CI 1.24-3.27; KPC: 2024 vs 2019, IRR 1.3, 95%CI 0.68-2.5). In TIBDN hospitals, indications for screening for CPE colonization as part of transmission control programs have been broadening over time. The proportion of patients first detected by a clinical specimen has declined from 80/147 (54%) from 2010-2014 to 199/551 (36%) from 2015-2019 and to 315/1263 (25%) from 2020-2024 ($p<0.0001$).

Conclusions: CPE infections are increasing in Toronto and Peel region, interrupted briefly by the pandemic. More effective control of in-hospital transmission is needed to combat this threat to public health.

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EpiScan – A geospatial health application for measles outbreaks Madinakhon Sulaymonova, Adan Amer, Bahja Farah, Chaandini Ranganathan, Fatima Ali

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Measles is a highly contagious viral disease that is a significant threat to communities with low vaccination rates. Although measles was declared eliminated in Canada in 1998, 2025 has seen the highest number of cases since then, with most occurring in Ontario. To address this public health crisis, the award-winning geospatial application EpiScan—winner of the 2025 Health GIS App Challenge hosted by Esri Canada and DLSPH—was developed to raise awareness, identify at-risk populations, and promote vaccine uptake. EpiScan informs the public about measles prevention through storytelling. It monitors measles cases and vaccination coverage in Ontario, identifies social and demographic predictors of outbreaks, estimates preventable cases in unvaccinated populations, and promotes vaccination with interactive tools and targeted messaging.

EpiScan was developed using ArcGIS tools using data from Public Health Ontario and Statistics Canada. The surveillance dashboards displayed measles case counts and rates, immunization rates, and demographic indicators by Public Health Unit (PHU). A proportional estimation of avoidable measles cases if all unvaccinated individuals had received one MMR dose was conducted.

Southwestern Public Health and Chatham-Kent Public Health reported the highest measles rates, at 181 per 100,000 and 120.3 per 100,000, respectively. PHUs with lower educational attainment, higher Anabaptist population proportions, and moderate visible minority representation showed higher case rates. 88% of current measles cases consist of unvacci nated individuals, however not all PHUs with high unvaccination rates have high measles case counts. The zero-dose modelling estimated that over 950 cases were avoidable (e.g. 333/429 (77.6%) in Southwestern Public Health).

EpiScan showcases the importance of geospatial tools for measles surveillance, risk communication, and public health planning. By integrating real-time data with demographic and vaccination information, the platform identifies high-risk areas and measures the impact of vaccine hesitancy. These insights inform targeted interventions and enhance immunization efforts to prevent future outbreaks in Ontario.

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Exploring the metabolism of *Neisseria gonorrhoeae* through genome-scale metabolic modelling

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Background: Neisseria gonorrhoeae is a host-adapted pathogen with a unique genome and metabolism which is capable of colonizing multiple diverse body sites despited its limited metabolic capacity. This combination may lead to enzymes becoming conditionally essential for gonococcal infection, which can then be targeted for developing new therapeutics. Given the challenges of testing gene essentiality across multiple conditions, in silico models such as GEnome scale Metabolic models (GEMs) can efficiently simulate gonococcal metabolism across diverse, biologically relevant sites and determine essential and conditionally essential genes.
Aim/Methods: We have constructed a new model of gonococcal metabolism through enzyme annotation and gap-filling, followed by incorporating insights from other models and available data of Neisseria metabolism. Additional genomic, transcriptomic, and proteomic data on the growth of prototypical strains is being incorporated to improve the accuracy of the simulations. We now aim to use this newly developed and refined model to predict conditional gene essentiality and obtain insights into gonococcal metabolism during infection.
Results: We have obtained an initial set of essential genes in minimal media which significantly overlap with previously identified essential gonococcal genes. We have also identified multiple instances of synthetic lethality through double gene knockouts, which may provide an alternative source of targets for combination therapies. We have begun the process of expanding the simulations to include conditions mimicking common sites of infection such as the cervicovaginal tract and the male urethra. Finally, we have observed an inhibition in bacterial growth when certain amino acids are removed from the growth media, hinting at a possible mechanism of nutritional immunity from the host.
Conclusions: The unique biology of N. gonorrhoeae may leave it vulnerable to the targeting of genes which are essential only to gonococcal colonization and survival. Metabolic modelling is a powerful tool to rapidly identify these genes in a condition-specific manner. Insights from our metabolic model will help develop new therapeutics and improve our understanding of gonococcal metabolism during infection.

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Profiling 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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Cellular and viral miRNAs are thought to play important roles in regulating Epstein-Barr virus (EBV) latent and lytic infections, 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, the main sites of lytic infection, we conducted miRNA-sequencing 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. 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 have pro-viral effects, as inhibiting all three together reduced EBV lytic protein expression. Interestingly, these miRNAs all originate from the DLK1-DIO3 locus (14q32.1 - 32.31), which encodes multiple lncRNAs. We showed that the lncRNAs MEG9, MIR381HG, and MEG8, from which miR-409-3p, miR-381-3p and miR-370-3p are derived, were also upregulated upon reactivation in AGS and nasopharyngeal carcinoma cells lines and occurred very early in the lytic cycle at the time of BZLF1 expression. In keeping with this timing, BZLF1 was sufficient to induce these lncRNAs dependent on its transactivation activity, and was detected at a key DLK1-DIO3 control element, consistent with a direct role in transcriptional activation. Therefore, we have identified a new role for BZFL1 in activating the expression of lncRNAs in the DLK1-DIO3 locus, resulting in induction of a subset of encoded miRNAs that promote lytic infection.