UNIVERSITY OF TORONTO

Microbiology & Infectious Diseases Research Days

Monday, June 3rd, 2019 – Trainee Day (Selected from Abstracts)
Tuesday, June 4th, 2019 – Invited Lectures & Poster Session

Talks in Medical Sciences Building, Room 2170

Posters & Lunch in Medical Sciences Building, Room 2171 (C. David Naylor Student Commons)

Website: http://microbeto.ca/mid-2019/

Monday, June 3rd, 2019

9:30 - 9:40 WELCOME ADDRESS

9:45 – 10:00: Avid Mohammadi
Characterizing the impact of penile-vaginal sex on HIV-susceptible CD4+ T cell subsets in the female genital tract

10:05 - 10:20: Erin O. Y. Wong
Developing defined microbiota to model inflammation in the mouse gut

10:25 - 10:40: Nora Mellouk
An ATG16L1-dependent pathway promotes plasma membrane repair and limits Listeria monocytogenes cell-to-cell spread

10:45 - 11:15: COFFEE BREAK

11:20 - 11:35: Jean-Paul R. Soucy
Joint modelling of resistance to six antimicrobials in urinary *Escherichia coli* isolates in Quebec, Canada

11:40 – 11:55: Sarah Birstonas
EHEC utilizes two-component systems to modulate expression of major flagellar subunit protein, FliC, in response to host intestinal cues

12:00 - 12:15: Nathaniel Winsor
NLRP6 regulates the colonic mucus layer during Tritrichomonas infection

12:35 – 1:30: LUNCH

1:35 - 12:50: Samuel Salamun
Epstein-Barr Virus Protein BMRF1 Modulates Cellular SUMO and DNA Damage Response Pathways by Binding the Cellular NuRD Complex

1:55 - 2:10: Nicola Case
Elucidating the mechanism of Candida albicans morphogenesis in response to phagocytosis by macrophages

2:15 - 2:30: Sarah Kronheim
A small molecule anti-phage defense mechanism in Streptomyces

2.30 - 3:00: COFFEE BREAK

3:05 - 3:20: Alexandra Willis
Understanding inherited immunity using a *C. elegans* model of microsporidia infection

3:25 - 3:40: Genevieve Mailhot
Differentiating between protective and pathogenic neutrophil responses during *Neisseria gonorrhoeae* infection

3:45 – 4:00: Tiffany Fitzpatrick
Successes of anti-RSV prophylaxis among infants in Ontario: results from a multi-decade, population-based controlled interrupted time series analysis using health administrative data

Tuesday, June 4th, 2019

8:00 AM REGISTRATION

8:20 - 8:30 WELCOME ADDRESS
Dr. Rupert Kaul & Dr. Scott Gray-Owen

8:30 - 9:00: Dr. Philip Lam
Physician Lead, Antimicrobial Stewardship in CMH, Sunnybrook Health Sciences Centre

Improving Cefazolin Use as a Pre-operative Prophylaxis in Patient with Beta-Lactam Allergy

9:00 - 9:30: Dr. Alon VAISMAN

Division of Infectious Diseases, University of Toronto
Infection Prevention and Control, Epidemiologist, University Health Network

After the Audit: Estimating the Residual Hawthorne Effect

9:30 - 10:00: Dr. Vanessa Allen
Chief, Medical Microbiology, Public Health Ontario
Assistant Professor, Department of Laboratory Medicine & Pathobiology

The Re-Emergence of Disseminated Gonococcal Infection

10:00 - 10:30: Dr. Peter Roy
Professor & Vice Chair, Department of Molecular Genetics, Department of Pharmacology & Toxicology, University of Toronto

Developing New Compounds to Fight Parasitic Nematode Infections

10:30 - 11:00: COFFEE BREAK

11:00 – 11:30: Dr. Karen Maxwell
Assistant Professor, Department of Biochemistry, University of Toronto

A Chemical Defence Against Phage Infection

11:30 - 12:00: Dr. Vincent Piguet
Department Division Director, Dermatology, University of Toronto & Division Head, Dermatology, Women's College Hospital

HIV mucosal transmission and skin manifestations of HIV

12:00 - 1:00: KEYNOTE ADDRESS
Dr. Guillaume Duménil
Head, Unité Pathogenèse des Infections Vasculaires, Institut Pasteur, Paris, France

Neisseria meningitidis vascular colonization

1:00 – 1:15: Closing Remarks and Awards

1:15 – 2:00: LUNCH

2:00 – 3:00 POSTER SESSION A
3:00 – 4:00 POSTER SESSION B

Thank you to the following sponsors who provided unrestricted educational funds to support this event:

Oral Presentations

1) Characterizing the impact of penile-vaginal sex on HIV-susceptible CD4+ T cell subsets in the female genital tract

Avid Mohammadi

Background: HIV in women is often acquired across the female genital tract mucosa, and a key parameter determining mucosal HIV susceptibility is the density of HIV-susceptible CD4+ T cells, particularly activated CD4+ T cells and Th17 cells. However, although most HIV transmission occurs during sex, the impact of sex itself on CD4+ T cell subsets is poorly described.

Methods: STI-free heterosexual couples (N=40) were recruited. Blood, cervico-vaginal secretions and a cervical cytobrush were collected from the female partner at baseline; couples then had penile-vaginal sex 48h later, with repeat sampling after 1-2 hr and 72 hr. Couples either had unprotected sex (n=31) or condom-protected sex (n=11); two couples participated twice, once with and once without a condom. Cytobrush-derived CD4+ T cell subsets were assessed by flow cytometry, and paired changes assessed by Wilcoxon signed-rank test.

Results: The proportion of endocervical Th17 (CCR6+) cells transiently increased 1-2 hr after penile-vaginal sex (median increase = 4.95%; p=0.008), and returned to baseline by 3 days. Endocervical activated (HLA-DR+) CD4+ T cells also increased after 1-2 hr, but these increases persisted for □72 hr (1.63%; p= 0.008 and 4.75%; p< 0.0001, respectively). Importantly, increases in both types of HIV target cells were only apparent after condomless sex (5.0% for CCR6; P=0.015 and 2.11% for HLA-DR; p=0.006), with no increase seen after condom-protected sex (1.1% for CCR6; 0.7% for HLA-DR; both p>0.3). The expression of CCR5 and the frequency of other cervical CD4+ T cell subsets, including Th1 and Trm, were unchanged after sex.

Conclusion: Penile-vaginal sex rapidly increased the proportion of cervical Th17 cells and activated CD4+ T cells, thought to be key endocervical CD4+ T cell HIV targets. Future work will assess the impact of sex on genital cytokine levels and the microbiota, and correlate cervical immune changes with semen parameters.

2) Developing defined microbiota to model inflammation in the mouse gut

Erin O. Y. Wong, Sudipta Saha, Emma J. E. Brownlie, William W. Navarre

Inflammation within the gastrointestinal tract is involved in the development of a wide array of gastrointestinal diseases such as colon cancer and inflammatory bowel disease. High throughput sequencing data has consistently shown that the composition of the gut microbiota varies dramatically between the inflamed gut and the non-inflamed gut. Mouse models are extensively used to investigate the interaction between the gut microbiota and inflammation, however much of the mouse microbiota remains uncharacterized. This fundamentally restricts both the amount of biologically relevant information that can be extracted, and our ability to utilize sequence data to its full potential. In particular, the lack of available isolates originating from the mouse gut limits the field from testing functional relationships. To address these issues, increasing efforts have been dedicated to characterizing and isolating the core microbiota of healthy mice. Little has been done to characterize and isolate the members of inflamed gut microbiota.

We have isolated and assembled a collection of mouse gut bacteria from mice with colitis. Our collection consists of over 52 strains representing 50 different species, including 13 novel species encompassing 7 novel genera and one novel family. 10 of these isolates are significantly more abundant in inflamed mice when compared with healthy mice from the same genetic background. We have sequenced the genomes of these strains and are beginning in vivo studies assessing colonization in germ-free mice and development of inflammation in genetically susceptible mice.

These strains and their genomes will be made publicly available to the scientific community. Strains in this collection can be used to design model ecosystems to investigate the role of the gut microbiota in the development of inflammation. It will also provide the ability to perform functional studies to assess the causal roles of key isolates and the corresponding mechanisms used to mediate inflammation.

3) An ATG16L1-dependent pathway promotes plasma membrane repair and limits Listeria monocytogenes cell-to-cell spread

Nora Mellouk 1*, Joel M.J. Tan 1,2*, Suzanne E. Osborne 1, Dustin A. Ammendolia 1,2, Diana N. Dyer 1,2, Ren Li 1, Diede Brunen 3, Jorik M. van Rijn 3, Ju Huang 1, Mark A. Czuczman 1,2, Marija Cemma 1,2, Amy M. Won 2, Christopher M. Yip 2, Ramnik J. Xavier 4, Donna A. MacDuff 5, Fulvio Reggiori 3, Jayanta Debnath 6, Tamotsu Yoshimori 7, Peter K. Kim 1,2, Gregory D. Fairn 2,8, Etienne Coyaud 9, Brian Raught 2,9, Aleixo M. Muise 1,2, Darren E. Higgins 4, John H. Brumell

Plasma membrane integrity is essential for the viability of eukaryotic cells. In response to bacterial pore-forming toxins, disrupted regions of the membrane are rapidly repaired. However, the pathways that mediate plasma membrane repair are unclear. Here we show that autophagy related (ATG) protein ATG16L1 and its binding partners ATG5 and ATG12 are required for plasma membrane repair through a pathway independent of macroautophagy. ATG16L1 is required for lysosome fusion with the plasma membrane and blebbing responses that promote membrane repair. ATG16L1 deficiency causes accumulation of cholesterol in lysosomes that contributes to defective membrane repair. Cell-to-cell spread by Listeria monocytogenes requires membrane damage by the bacterial toxin listeriolysin O, which is restricted by ATG16L1-dependent membrane repair. Thus, plasma membrane repair could be an important therapeutic target for the treatment of bacterial infections.

4) Joint modelling of resistance to six antimicrobials in urinary *Escherichia coli* isolates in Quebec, Canada

Jean-Paul R. Soucy, Alexandra M. Schmidt, Charles Frenette, Patrick Dolcé, Alexandre A. Boudreault, David L. Buckeridge, Caroline Quach

Introduction: Empirical treatment of urinary tract infections should be based on susceptibility profiles specific to the locale and patient population. Additionally, these susceptibility profiles should account for correlations between resistance to different types of antimicrobials, rather than treating each type of resistance as independent.

Methods: We used hierarchical logistic regression models to investigate geographic, temporal, and demographic trends in resistance to six antimicrobials in community-acquired and nosocomial urinary E. coli isolates from three communities in the province of Quebec, Canada, procured between April 2010 and December 2017.

Results: A total of 74,986 community-acquired (patient age, ≥18 years) and 4,384 nosocomial isolates (patient age, ≥65 years) were analyzed. In both community-acquired and nosocomial isolates, we found geographic variation in the prevalence of resistance. Male sex (community-acquired hierarchical mean odds ratio [OR], 1.24; 95% credible interval [CI], 1.02 to 1.50; nosocomial hierarchical mean OR, 1.16; 95% CI, 0.92 to 1.41) and recent hospitalization (community-acquired hierarchical mean OR, 1.49; 95% CI, 1.33 to 1.66; nosocomial hierarchical mean OR, 1.31; 95% CI, 0.99 to 1.78) were associated with a higher risk of resistance to most types of antimicrobials. We found distinct seasonal trends in both community-acquired and nosocomial isolates, but only community-acquired isolates showed a consistent annual pattern. Ciprofloxacin resistance increased sharply with patient age.

Conclusions: We found clinically relevant differences in antimicrobial resistance in urinary E. coli isolates between locales and patient populations in the province of Quebec. These results could help inform empirical treatment decisions for urinary tract infections. In the future, similar models integrating local, provincial, and national resistance data could be incorporated into decision support systems for clinicians.

5) EHEC utilizes two-component systems to modulate expression of major flagellar subunit protein, FliC, in response to host intestinal cues

Sarah Birstonas1, Debora Barnett Foster1,2

Enterohemorrhagic Escherichia coli (EHEC) O157:H7 is a food- and water-borne human enteric pathogen that can cause severe hemorrhagic colitis and may lead to hemolytic uremic syndrome (HUS) which can be fatal. In order to effectively colonize the human gastrointestinal tract, EHEC uses virulence factors including flagella to reach its site of colonization in the large intestine and infect host epithelial cells. Expression of flagella, along with numerous other virulence factors can be modulated in response to microenvironmental conditions within the gastrointestinal tract as sensed by bacterial two-component systems (TCS). We have previously demonstrated that short chain fatty acid (SCFAs) mixes representative of small intestinal levels upregulate FliC expression while mixes representative of large intestinal levels downregulate FliC expression. Here we show that flagella expression is also sensitive to oxygen and bicarbonate levels in combination with nutrient levels of media and SCFAs. To evaluate the role of selected TCSs in these differential responses, we constructed three response regulator mutants, previously shown to affect the SCFA-induced flagella expression phenotype in non-pathogenic E. coli, - arcA, rcsB and uvrY. These isogenic EHEC mutants were assayed for FliC expression under different SCFA mixes alone and in combination with the other intestinal cues including low oxygen and physiologically relevant bicarbonate levels. Distinctive differences in FliC expression profiles were evident with a loss of the SCFA wild type phenotype linked to the arcA mutation. The changes in the expression profile of ΔrcsB, and ΔarcA compared to wild type indicate responses in flagella expression are partially dependant on sodium bicarbonate and differential oxygen conditions respectively. Collectively, these results demonstrate that expression of flagella, which play a key role in mediating motility, are acutely sensitive to host environmental conditions in ways that generate synergies with different treatment combinations.

6) NLRP6 regulates the colonic mucus layer during Tritrichomonas infection

Nathaniel Winsor (1), Paul Lemire (2), Samuel Killackey (2), Susan Robertson (1), Heather Maughan (3), Ana Popovic (4), John Parkinson (4), Dana Philpott (1), Stephen Girardin (1,2).

Pathogen detection often involves members of the NOD-like receptor (NLR) family of proteins, which interact with adaptor and caspase proteins to form complexes known as inflammasomes. By activating cytokines such as IL-18, inflammasomes serve as key mediators of inflammatory signaling. NLRP6 is the most abundant NLR protein in intestinal epithelial cells (IECs), yet the processes governing its activation and subsequent signalling are unclear. Reports that colorectal cancer (CRC) is exacerbated in protozoan-infected animals prompted us to explore the role of NLRP6 in Tritrichomonas (Tm) protist infection, and tumorigenesis. Using the genetic Apcmin/+ model of CRC, we determined that chronically infected Nlrp6-/- Apcmin/+ mice have a greater intestinal tumour burden than Apcmin/+ littermates. We excluded a role for the microbiome, as regardless of Tm infection, 16S rDNA sequencing of bacterial communities in Nlrp6-/- Apcmin/+ and Apcmin/+ mice did not reveal significant differences. It has been suggested that NLRP6 regulates both goblet cell abundance and mucus secretion. While we found no NLRP6 dependent difference in goblet cell levels or morphology, Tm infected Nlrp6-/- mice possess a thinner mucus layer, suggesting that NLRP6 regulates mucus secretion in response to pathogenic stimulus. Moreover, we found that IL-18 is sufficient to induce mucus secretion ex vivo, suggesting a potential role for the NLRP6 inflammasome in regulating the colonic mucus layer. Future work aims to determine whether the NLRP6-mucus axis is specific to enteric parasites or represents a more general response to infection.

7) Epstein-Barr Virus Protein BMRF1 Modulates Cellular SUMO and DNA Damage Response Pathways by Binding the Cellular NuRD Complex

Samuel Salamun, Carlos De La Cruz-Herrera, Edyta Marcon, Jack Greenblatt, Lori Frappier

Epstein-Barr virus (EBV) infects approximately 90% of the adult population worldwide, and is associated with several types of cancers, including Burkitt's lymphoma, nasopharyngeal carcinoma, and gastric carcinoma. EBV encodes approximately 80 proteins, many of which function to manipulate cellular processes to promote cell survival and viral infection. However, the functions of many EBV proteins are poorly characterized or completely unknown. The Frappier lab screened a library of EBV proteins to identify those that alter SUMO (Small Ubiquitin-like Modifier) modifications of cellular proteins, and those that inhibit the cellular DNA damage responses (DDR) that would normally be triggered by the linear dsDNA genome of EBV and would lead to apoptosis. The EBV protein BMRF1 was found to upregulate global SUMOylation, and to inhibit DDR signaling in response to dsDNA breaks. BMRF1 has known roles in viral replication as the viral DNA polymerase processivity factor (sliding clamp) and in activating the transcription of some EBV genes. However, its roles in manipulating cellular processes, such as SUMOylation and DNA damage responses, have not been previously examined.

To understand the mechanism by which BMRF1 alters cellular processes, we used affinity purification coupled to mass spectrometry to profile the host protein interactions of FLAG-tagged BMRF1 in human cells. This showed that all 14 subunits of the cellular nucleosome remodeling and deacetylation (NuRD) complex co-purified with BMRF1. The chromatin remodeling ability of NuRD is known to both activate and repress transcription of different genes and to be an important step in the DDR to dsDNA breaks. Interactions between NuRD and some cellular proteins are mediated by an RKKQxxP motif, which allows binding to the RBBP4 subunit. We have identified this motif on the C-terminus of BMRF1, point mutated it and confirmed that the mutations abrogate the BMRF1-NuRD interaction. In addition, mutations in this motif in BMRF1 abrogated the induction of cellular SUMOylation, suggesting that BMRF1 induces SUMOylation by binding NuRD and altering gene expression. We also examined the steps in the DDR that are inhibited by BMRF1 and showed that BMRF1 interfered with ubiquitylation at the dsDNA breaks, which is the same step known to be regulated by NuRD. In keeping with this result, the BMRF1 mutants disrupted in NuRD binding were less able to inhibit the DDR. Together, our findings indicate that BMRF1 modulates both the DDR and SUMOylation through interactions with NuRD, and thus demonstrate novel interactors and functions of a sliding clamp protein.

8) Elucidation of the mechanism of *Candida albicans* morphogenesis in response to phagocytosis by macrophages

Nicola Case1, Kwamaa Duah1, Teresa O'Meara1, Brett Larsen3, Cassandra J. Wong1,3, Sang Hu Kim1, Robert T. Todd2, Amanda O. Veri1, Anne-Claude Gingras1, 3, Anna M. Selmecki2, & Leah E. Cowen1

Macrophages are among the first line of defense against invasive fungal infections. While typically successful at killing foreign invaders, the human fungal pathogen, Candida albicans, is equipped with elegant mechanisms that allow it to subvert this fate. In C. albicans, engulfment by macrophages triggers a morphogenetic transition from yeast to hyphal growth, which induces macrophage cell death and leads to fungal escape. The genetic circuitry underlying filamentous growth has been studied in response to the inducing cue serum. However, the signaling pathways that coordinate filamentation in response to other host-relevant cues remain largely enigmatic. Furthermore, studies have hypothesized that C. albicans filamentation is induced as a result of changes in pH, nutrient deprivation, or oxidative stress experienced in the macrophage phagosome. However, we recently discovered that macrophage lysate is sufficient to induce C. albicans morphogenesis, indicating other macrophage-specific factors are at play. Bioactivity guided fractionation coupled to mass spectrometry identified the known immune modulator, prothymosin alpha (PTMA), as a potential macrophage-derived trigger of filamentation. Immunodepletion of PTMA from macrophage lysate abolished its ability to induce C. albicans filamentation, supporting PTMA as a filamentation inducing component in macrophage lysate. To identify the genes that orchestrate filamentation in the macrophage, we performed a functional genomic screen of conditional expression mutants covering ~40% of the C. albicans genome and identified 298 genes important for filamentation upon internalization by macrophages. Notably, fifty-four genes had no defect in response to serum, demonstrating specificity in the circuitry governing morphogenesis within macrophages. Initial characterization of nineteen of these mutants identified a subset that were defective in filamentation in response to macrophage lysate, suggesting multiple macrophage-derived factors trigger distinct fungal response circuitry. Overall, this work identifies a macrophage-specific trigger of filamentation and establishes novel regulatory circuitry governing fungal morphogenesis in response to a host immune cell.

9) A small molecule anti-phage defense mechanism in Streptomyces

Sarah Kronheim, Martin Daniel-Ivad, Renee Duan, Andrew Wong, Ian Mantel, Justin R Nodwell, Karen L Maxwell

Viruses that infect bacteria are known as bacteriophages, or phages. They are among the most abundant and diverse entities on the planet. Phages outnumber bacteria roughly tenfold and are responsible for the death of approximately 20-40% of prokaryotic biomass each day, significantly impacting the composition and diversity of microbial communities. As a result of this evolutionary pressure, bacteria have developed numerous defense mechanisms to protect themselves from phage infection. Given the diversity of known defense mechanisms and the continual evolutionary battle between phages and their host bacteria, additional uncharacterized defense mechanisms likely exist.

Streptomyces are filamentous bacteria with extensive secondary metabolisms that produce many bioactive compounds, including roughly two-thirds of clinically relevant antibiotics. Several natural products produced by Streptomyces spp. have been shown to inhibit the activity of phage lambda, which infects Escherichia coli. In this work we examine the ability of small molecules produced by Streptomyces peucetius and other Streptomycetes to inhibit phage activity. In particular, we explore this ability in DNA intercalators produced by Streptomyces, small molecules that insert into the base pairs of DNA. In conclusion, the production of small molecules with anti-phage activity appears to be a novel phage defense mechanism utilized by Streptomyces bacteria.

10) Understanding inherited immunity using a *C. elegans* model of microsporidia infection

Alexandra Willis

It has recently emerged that some animals can pass on ‘memory’ of previous infection to their offspring, thereby enhancing immunity their progeny. This 'inherited immunity' is an example of epigenetic inheritance, whereby the parental environment modulates gene expression in the germline, without altering the DNA sequence. Though inherited immunity can provide resistance to bacterial, viral and fungal pathogens, the molecular mechanisms underlying protection are unknown.

Studies in C. elegans have enabled key advances in both immunity and epigenetics. Microsporidia are poorly-characterised fungal pathogens, and Nematocida parisii is a microsporidial parasite of C. elegans. New data shows that the progeny of N. parisii-infected worms exhibit robust immunity against microsporidia. Whilst microsporidia replicate in the intestinal cells of naïve larvae and inhibit worm development, parasitism is absent in our immune-primed larvae. These striking phenotypes make the N. parisii-C. elegans infection model an ideal system to uncover mechanisms of inherited immunity.

We are now fully characterising our inherited immunity phenotypes in vivo. In particular, studies of parasite invasion and clearance are revealing mechanisms of protection in resistant worms, and gene expression analyses are being used to reveal putative immune effectors. We are also assaying epigenetic factors to better understand the mechanism of immune transmission from parent to progeny.

This study offers unique insight into the immune reactivity of the genome. Epigenetic factors mediating inherited immunity in C. elegans may have homologues in higher organisms; inherited immunity in vertebrates is unexplored and would have major implications for human health and evolution. Whilst microsporidia are important opportunistic pathogens of humans and parasitize many agriculturally important species, very little is known of their infection biology. Study of this medically and environmentally relevant pathogen will suggest new ways to treat infection.

11) Differentiating between protective and pathogenic neutrophil responses during *Neisseria gonorrhoeae* infection

Genevieve Mailhot, Scott Gray-Owen

Background: Neisseria gonorrhoeae (Ng) is the human-restricted bacterial pathogen responsible for the sexually transmitted infection, gonorrhea. Untreated infection in women can often persist, leading to devastating sequelae such as pelvic inflammatory disease (PID). In some cases, however, infection clears before causing any clinical symptoms, even without antibiotic intervention. Moreover, an adaptive immune response does not develop to protect against subsequent infections. These observations raise questions as to what host immune factors contribute to immune protection versus immuno-pathogenesis.

Methods: Here, I exploit a mouse-based gonococcal infection model of the upper genital tract to study the early immune response to Ng. To reveal the earliest responders to Ng, I utilize a transgenic mouse line with cells that express the green fluorescence protein (GFP) upon the activation of the immediate early transcription factor, NF-κB, which is rapidly upregulated upon innate recognition of microbial infection. I also utilize a gonococcal fluorescent stain to visualize which cells associate with Ng during early infection.

Results: My results demonstrate a massive recruitment of neutrophils into the infected uterus peaking at 6 hours post-infection, with only a subset of these cells activating NF-κB. Additionally, fluorescent staining of Ng reveals that the majority of Ng-associated cells are neutrophils, most of which do not appear to activate NF-κB.

Discussion: These data make it enticing to consider that these different neutrophil responses reflect diverse neutrophil phenotypes, since recent work indicates that neutrophils may function to either promote or suppress immune function. Continued work will establish the role of these diverse neutrophil populations in the context of gonococcal infection. This work will shed light on how the early immune response may be contributing to the development of Ng-induced PID so that they can be circumvented or suppressed.

12) Successes of anti-RSV prophylaxis among infants in Ontario: results from a multi-decade, population-based controlled interrupted time series analysis using health administrative data

Tiffany Fitzpatrick, Drew Wilton, Thérèse Stukel, Dayre McNally, Jeff Kwong, David Fisman, Astrid Guttmann

Background: Respiratory syncytial virus (RSV) is the leading cause of lower respiratory infections and hospitalization among children worldwide. While there are yet no licensed vaccines, RSV prophylaxis (Palivizumab) is available to children at increased risk of severe RSV-related illness. In Ontario, Palivizumab has been publicly funded for high-risk infants since 2002 and through a national special access program since 1998.

Objective: to evaluate changes in severe pediatric RSV-related illness (admissions, deaths) following the introduction of Palivizumab in Ontario. We further investigated potential differences across socio-economic factors.

Methods: All infants born in Ontario Jan 1st, 1993 through Dec 31st, 2016 were followed until the earliest of: 2nd birthday; death; moving out of province; or end of follow-up, June 30th, 2017. All hospital admissions for bronchiolitis, RSV pneumonia, or flagged with an RSV diagnosis and all RSV-related deaths were identified using linked administrative health data. High-risk infants were defined according to program guidelines identifiable through administrative health data. Interrupted time series analyses were conducted to assess changes in pre- vs. post-program rates and trends. Differences across neighbourhood income quintiles, rurality, and maternal age were also investigated. All analyses were stratified by age at infection (<6, 6-12, 12-24 months).

Results: Over 3 million infants and nearly 87,000 admissions were identified. RSV burden was substantially greater among infants <6 months. Statistically significant declines were seen among all <6-month olds following both programs (1998: $p=0.0167$, 2002: $p<0.0001$). Rates markedly dropped among high-risk infants over time (60%). Even within high-risk infants, rates were consistently greater among children from low income areas and born to teenage mothers; however, these gaps substantially narrowed over time.

Conclusions: This large, population-based study of infants spanning 24-years found significant declines in severe RSV-related illness (hospital admissions, deaths) following the introduction of palivizumab in Ontario, along with evidence to suggest narrowing social inequities.

Poster Presentations

1) Could reducing time to bacterial identification from positive blood cultures improve outcomes in bacteremic patients?

Jessica D Forbes1, Reem Haj2, Linda R Taggart2,3, Ramzi Fattouh1,2, Elizabeth Leung2, Jan Freidrich2, Larissa M Matukas1,2,3

1Department of Laboratory Medicine & Pathobiology, University of Toronto 2St. Michael's Hospital, Toronto, Ontario 3Divison of Infectious Diseases, Department of Medicine, University of Toronto

Introduction

Survival of patients with septic shock is dependent on the timing of effective antibiotic administration. The initial notification by the microbiology lab of a positive blood culture is a key factor in improving patient outcomes. It can take >24 hours to definitively identify bacteria from positive blood cultures. Accordingly, we employed rapid organism identification and studied the impact of this on patient management from a quality improvement perspective.

Methods

Rapid organism identification was performed for bacteremic patients admitted to an ICU at St. Michael's Hospital in Toronto, ON, by creating a pellet from positive blood culture bottles using a lysis centrifugation technique. MALDI-TOF was then used to obtain an organism identification. The microbiology lab verbally notified the ward clerk of the identification and surveys were conducted with treating physicians within 24-48 hours to evaluate the downstream impact of the rapid identification including changes to antibiotics, diagnostic testing, central line management and requests for specialty consultations.

Results

Between January 28–April 28, 2019, 17 rapid blood culture results were included for study. When asked how physicians received the result, in 7 cases the physician did not remember; other responses included microbiology report (2), nurse (2), pharmacist (1), antimicrobial stewardship or lab (1), on call team (1) and residents (1). Antibiotics were adjusted in 13 patients; 3 of which may have changed antibiotics for reasons other than the organism identification. Reasons for not changing therapy include: appropriate empiric treatment, likely contaminants, or physician not being notified of the result. In 5 cases, all antibiotics were discontinued, in another 2 cases the antibiotics were broadened and a further 5 narrowed to cover the organism; the remaining 5 continued the same empiric therapy. Repeat blood cultures were obtained for 5 cases, follow-up imaging in 5 cases and lines were changed/removed in 5 cases. Consultation was requested for 7 cases.

Conclusions

Based on preliminary data, rapid organism identification shows promise of improved patient management with line removal and antibiotics adjustments occurring 1 day sooner with rapid results.

2) Retrospective Evaluation of the Effectiveness of Fecal Microbiota Transplantation on Antibiotic Resistant Organism Clearance – A Pilot Study

Jordan Fruitman, Melissa Kissoon, Jessica Forbes, Samuel Fung, Susy Hota, Susan M. Poutanen

UofT Microbiota Therapeutics Outcomes Program (MTOP), department of microbiology at SHS

Background: Patients exposed to broad-spectrum antibiotics have depleted commensal bacteria and increased risk of antibiotic resistant organisms (ARO) colonization. Fecal microbiota transplantations (FMT) restores the gut microbiome by transplanting healthy donor's microorganisms. Preliminary data suggests FMT may help decolonize AROs through competition with susceptible organisms.

Objectives of Study: To evaluate the effectiveness of FMT by enema and to determine the number of FMTs required for ARO eradication.

Methods: 12 MTOP patients treated with FMT by enema for rCDI and 7 patients treated with FMT from a rCDI RCT who had pre- and post-FMT stool or rectal swabs were investigated. Culture-based ARO screening was conducted. Bacteria were identified via MALDI MS (bioMérieux®). ESBL production was confirmed using clover-leaf inhibition method; meropenem non-WT Gram-negative bacteria were tested with βCARBA (Bio-Rad®) to confirm CPO; VRE was confirmed using Xpert-vanA/B PCR (Cepheid®); MRSA was confirmed using DENKA (Seiken®). ARO clearance was screened between FMTs from rectal swabs, and post-FMT from stool. Microbiota diversity was determined using 16s rRNA sequencing. Correlation with clinical outcomes was completed.

Results: 5/19 patients had AROs detected pre-FMT (VRE n=2; ESBL n=2; VRE and ESBL n=1). 2/5 [95%CI: 0.12-0.77] of patients had complete ARO clearance post-FMT. 2/3 [95%CI: 0.20-0.94] of ESBL colonized patients and 1/3 [95%CI: 0.06-0.80] of VRE colonized patients had ARO clearance post-FMT. 2/3 [95%CI: 0.20-0.94] of patients whose rCDI symptoms were cleared, had ARO eradication. 2/2 [95%CI: 0.29-1.00] of patients with sustained uptake of donor microbiota, eradicated their AROs post-FMT. All rectal swab screening between FMTs were negative.

Discussion/Conclusion: FMT has an estimated efficacy of 100% [95%CI: 0.29-1.00] in patients with sustained uptake of donor microbiota. It is challenging to interpret the optimal number of FMT delivered, given different sensitivities associated with stool cultures versus rectal swabs. Prospective evaluation of FMT on ARO eradication would be useful.

3) Safety and Effectiveness of a Laboratory Intervention to Reduce Antibiotic Consumption in Patients with Asymptomatic Bacteriuria

Mohammad Mozafarihashjin, Lorraine Maze Dit Mieusement, Allison McGeer, Liz McCreight, Liz Van Horne, Jannice So, Ananya Shrivastava, Nadeem Khan, Louis Wong, Jerome A. Leis

Infection Prevention and Control, Sinai Health System, Toronto, ON 2- Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON

Background: Antibiotic therapy for asymptomatic bacteriuria (ASB) persists despite evidence of lack of benefit. Following initial rejection of positive midstream urine (MSU), we moved to rejecting all MSU. We hypothesized that rejecting MSU from adult inpatients would reduce ASB antibiotic use and not cause serious adverse events (AEs).

Methods: From 11/2013 to 04/2019, when MSU were received from surgical and medical wards in our hospital, a message was posted noting that ASB should not be treated and a call to lab was required to initiate specimen processing. Patients were interviewed, and charts were reviewed within 24h of specimen receipt and 4d later to identify urinary tract symptoms/infection (UTS/UTI) and systemic infection. Primary outcome was AEs. Secondary outcomes were: rate of MSU submitted, impact on lab workload, antibiotic use.

Results: 1678 episodes with submitted MSU were included; 995/1678 (60%) MSU were not processed. Of 683 processed, 482 (71%) were negative. 1111/1678 (66%) patients were asymptomatic when MSU was ordered. 1393/1678 (83%) had negative culture (N=482) or completed d4 follow up (N=911). No symptomatic UTI/sepsis/systemic infection occurred; the only AE identified were 4 patients with prolonged UTS which might have been prevented by MSU processing (4/911; 0.4% pts with AE). Rates of MSU submitted remained stable at 12 per 1000 patient days (P=0.59). Processed MSU proportion decreased from 16/22, 73% in 2013 to 67/137, 49% in 2019 (P=0.002). Overall microbiology workload decreased by 5person-days/yr. 275/1678 (16%) patients received antibiotics for presumed UTI; 221 (80%) treated empirically, 54 (20%) in response to positive MSU. Of 69 patients with ASB, 32 (46%) were prescribed antibiotics. Assuming 21% of rejected MSU from asymptomatic patients would have been positive, antibiotic therapy for ASB was avoided in 66 patients.

Conclusion: Rejecting MSU specimens does not result in harm, and reduces lab workload and antibiotic therapy for ASB.

4) Healthcare-Acquired (HA) Carbapenemase-Producing Enterobacteriales (CPE) in Southern Ontario, Canada: To Whom are we Transmitting CPE?

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Dalla Lana School of Public Health, University of Toronto; Toronto Invasive Bacterial Diseases Network

Background: Though CPE in Canada are mainly acquired abroad, outbreaks/transmission in Canadian hospitals have been reported. We determined the incidence of HA CPE in southern Ontario, Canada, to inform prevention and control programs.

Methods: Toronto Invasive Bacterial Diseases Network (TIBDN) has performed population-based surveillance for CPE in the Toronto area/Peel region of southern Ontario, Canada, since CPE were first identified in Oct. 2007. Clinical microbiology laboratories report all CPE isolates to TIBDN; annual lab audits are performed. Incidence calculations used first isolates as numerator; denominator (patient-days/fiscal year for Toronto/Peel hospitals) was from the Ontario Ministry of Health and Long-Term Care.

Results: The incidence of HA CPE has risen from 0 in 2007/8 to 0.45 and 0.28 per 100,000 patient-days for all and clinical cases, respectively, in 2017/18 ($p < 0.0001$). 190/790 (24%) incident cases of CPE colonization/infection in southern Ontario from Oct. 2007-Dec. 2018 were likely HA (hospitalized in Ontario with no history of hospitalization abroad/high-risk travel). 80 (25%) were female and median age was 73 years (IQR 57-83 years). 157 (83%) had no prior travel abroad and 33 (17%) had prior low-risk travel. 122 (64%) had their CPE identified > 72 hours post-admission (of which 83 also had ≥1 other prior Ontario hospitalization); 68 (36%) had their CPE identified at admission but had recent prior Ontario hospitalization. HA cases vs. foreign acquisitions were significantly more likely K. pneumoniae (48% vs. 38%, $p=0.02$) and Enterobacter spp. (20% vs. 7%, $p < 0.0001$) and less likely E. coli (20% vs. 48%, $p < 0.0001$). Genes of HA vs. foreign acquisitions were significantly more likely blaKPC (34% vs. 12%, $p < 0.0001$) and blaVIM (12% vs. 2%, $p < 0.0001$) and less likely blaNDM±OXA (38% vs. 56%, $p < 0.0001$) and blaOXA (13% vs. 27%, $p=0.0001$). 36 (19%) HA cases had a negative CPE screen before their first positive CPE test (10/36 (28%) were on admission). The median incidence of HA CPE per 100,000 patient-days at each hospital was 0.44 (IQR 0.15-0.68) ($p < 0.0001$).

Conclusion: A quarter of CPE cases in southern Ontario were HA and the incidence of HA cases is increasing. Most cases were admitted to > 1 Ontario hospital. Strategies to control transmission are critical.

5) Carbapenemase-Producing Enterobacteriaceae (CPE) in Household Contacts and Household Environments of CPE-Colonized/Infected Persons

Alainna Jamal, Lubna Farooqi, Amna Faheem, Zoe Zhong, Irene Armstrong, Emily Borgundvaag, Brenda Coleman, Karen Green, Kithsiri Jayasinghe, Jennie Johnstone, Kevin Katz, Philipp Kohler, Angel Li, Roberto Melano, Matthew Muller, Sarah Nayani, Samir Patel, Aimee Paterson, Susan Poutanen, Anu Rebbapgrada, David Richardson, Alicia Sarabia, Shumona Shafinaz, Andrew Simor, Barbara Willey, Laura Wisely, Allison McGeer

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Objectives: CPE have emerged rapidly worldwide. This prospective cohort study aimed to assess the risk of CPE transmission from colonized patients (index cases, ICs) to household contacts (HCs).

Methods: ICs were identified by population-based surveillance. Groin and rectal swabs, and urine specimens were collected from ICs/HCs at home visits every 3 months; swabs of 10 environmental surfaces (ESs) were also collected. Swabs/specimens were incubated in BHI broth overnight, followed by direct PCR to detect carbapenemase genes and cultures of PCR-positive samples.

Results: 97 households and 179 HCs participated. 19 (11%) HCs in 19 (19%) households were CPE-colonized. 9/19 (47%) were CPE-colonized at first visit. The probability of initially non-colonized HCs becoming CPE-colonized by month 12 was 9% (Figure). Overall, 14/19 (74%) CPE-colonized HCs probably/possibly acquired CPE from the IC; 5 (26%) probably acquired CPE from travel. HCs were more likely to be older (1.4 per 10-year increase, 95% CI 1.1-1.8), be the IC's spouse (OR 15, 95% CI 4.3-56), have an underlying chronic medical illness (OR 5.9, 95% CI 2.2-16), and to use the same primary bathroom as the IC (OR 3.2, 95% CI 1.1-9). Households with CPE-positive ESs were more likely to have CPE-colonized HCs (OR 10.83, 95% CI 2.62-44.83). 322/3077 (11%) ES samples representing 210 unique ESs yielded CPE. CPE yield per ES type was: kitchen sink drain (19%), shower drain (18%), bathroom sink drain (17%), toilet drain (13%), pillow (12%), sofa/chair (11%), bathroom sink tap (9%), toilet handle (6%), telephone (5%), and kitchen sink tap (4%). Of 210 CPE-positive ESs, 67%, 21%, 8%, 3%, and 2% yielded CPE for 1, 2, 3, 4, and 5 visits, respectively.

Conclusions: After 12 months, transmission occurred to 9% of HCs of CPE-colonized patients. CPE contamination of household surfaces, particularly drains, is common.

6) Atg16l1 deficiency exacerbates acute small intestinal epithelial damage

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Polymorphisms of the autophagy-related gene ATG16L1 are known to contribute to the genetic risk for developing Crohn's Disease (CD), an inflammatory bowel disease that can affect the entire gastrointestinal tract. Previous studies have shown a role for autophagy in the intestinal epithelium both in Paneth cell function and in protection from intestinal damage, but no spontaneous disease at baseline. Here, we challenged mice specifically deleted for Atg16l1 in intestinal epithelial cells (Atg16l1ΔIEC) with the anti-CD3 mediated model of acute small intestinal injury. Rapid T cell activation by anti-CD3 injection results in small intestinal damage within 24 hours of injection which typically self-resolves in wild-type mice 5 days post treatment. We found that autophagy sufficiency protected against anti-CD3 induced injury. Atg16l1ΔIEC mice had higher mortality and worsened pathology in comparison to their wild-type littermates. At one day after treatment, Atg16l1ΔIEC mice had significantly increased apoptotic bodies, villous blunting, and depletion of goblet and Paneth cells. We found indications of increased barrier permeability and crypt cell apoptosis in Atg16l1ΔIEC mice. In addition, expression of interferon gamma (IFNγ) was significantly increased in Atg16l1ΔIEC mice, a finding that has been previously implicated in driving intestinal injury in autophagy-deficient mice. Pathology continued to be increased in Atg16l1ΔIEC mice five days after treatment. Intra-epithelial lymphocytes have been shown to be activated by anti-CD3 treatment leading to epithelial pathology. The baseline intra-epithelial lymphocyte (IEL) makeup was altered in Atg16l1ΔIEC mice with increased frequencies of CD8+ T cells and γδTCR+ T cells. This suggests a baseline difference in IELs may be driving the exacerbated response to anti-CD3 treatment in Atg16l1ΔIEC mice.

7) Investigating the Interactions Between Candida albicans, Lactobacillus sp. and the Host-Immune Response

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Interactions between bacteria and fungi are ubiquitous in nature, yet little is known about the details of these interactions or their therapeutic potential. In humans, the opportunistic fungal pathogen, Candida albicans, is a common member of the mucosal microbiota that is capable of causing both superficial infection and life-threatening systemic disease. Vaginal candidiasis occurs in approximately
75% of healthy women at least once in their lifetime, and previous work has demonstrated that fungal overgrowth often occurs following a decline in bacterial abundance that can result from antibacterial treatment and other factors. Lactobacillus species, prominent constituents of the vaginal microbial community, represent the most common industrial probiotic, and current research is focused on
exploiting probiotic bacterial species to promote a healthy vaginal microbiome. With the goal of identifying the mechanism(s) by which specific probiotic organisms affect C. albicans virulence, we examined the effects of Lactobacillus strains from Lallemand Health Solutions Inc. on the ability of C. albicans to switch from yeast to filamentous morphologies, a cellular transition important for virulence. We observed that several strains of Lactobacillus secrete a factor that is able to repress morphogenesis in C. albicans. Bioassay-guided fractionation and subsequent structural elucidation work was able to link this activity to a single molecular entity. Chemical synthesis of this compound additionally resulted in the production of a bioactive by-product of novel chemical origin. Future work aims to elucidate the mechanisms underlying this inter-kingdom interaction and facilitate the development of optimized probiotics and novel therapeutic strategies.

8) Leveraging Legionella pneumophila CRISPR-Cas to identify exogenous threats to pathogen survival

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Legionella pneumophila is an accidental pathogen that primarily infects freshwater protozoa. Exposure to the pathogen, in the form of contaminated aerosols, can lead to a severe, often fatal pneumonia - Legionnaires' disease. As there is no human-to-human spread of Legionella, understanding these bacteria's replication and persistence in environmental reservoirs is critical to limiting their ability to cause human disease. CRISPR-Cas can provide a window into this biology.

Endogenous CRISPR-Cas systems are rich genomic diaries of past environmental challenges (e.g. from bacteriophages and other foreign genetic elements). Our lab previously demonstrated that type I-F and type I-C CRISPR-Cas systems are active and adaptive in L. pneumophila, making them excellent repositories of past environmental encounters. Through spacer homology, we discovered the first known target of L. pneumophila CRISPR-Cas: an episome we called Legionella mobile element-1 (LME-1). We have subsequently shown that carriage of LME-1 restricts L. pneumophila host range, a critical determinant of environmental fitness.

With the recent availability of several hundred additional L. pneumophila genomes, we have embarked on a project to extensively catalog the spacers present within L. pneumophila CRISPR-Cas systems. Thus far, we have surveyed 147 CRISPR-Cas systems (across 523 L. pneumophila genomes) and identified 1590 unique spacer sequences. Using a BLAST-based pipeline, we have already identified several additional alleles of LME-1, which remains the only known target of L. pneumophila CRISPR-Cas. To find more targets, we are querying several sequence databases. We will present the results of these searches and discuss the downstream functional assays that they inform.

9) Identification of ARKL1 as a Jun-interacting Factor that Negatively Regulates Epstein-Barr Virus Reactivation

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Epstein-Barr virus (EBV) is a widespread herpesvirus that is associated with gastric and nasopharyngeal carcinomas and several types of lymphomas. EBV alternates between latent and lytic modes of infection, allowing it to persist for the life of the host. Reactivation from the latent to the lytic cycle starts with activation of the Z promoter (Zp) controlling expression of the EBV BZLF1 transcription factor. Zp includes a binding site for c-Jun which was previously shown to be an important activator of Zp. We identified the cellular Arkadia-like 1 (ARKL1) protein as a negative regulator of Zp and EBV reactivation. Silencing of ARKL1 in EBV-positive gastric carcinoma, nasopharyngeal carcinoma and B cells resulted in BZLF1 expression and EBV reactivation, while overexpression of ARKL1 decreased EBV reactivation. To gain insight into the mechanism of action of ARKL1, we isolated FLAG-tagged ARKL1 from human cells and identified interacting proteins by tandem mass spectrometry. This confirmed an interaction we identified previously between ARKL1 and CK2 kinase, and also revealed an interaction of ARKL1 with Jun, suggesting that ARKL1 might suppress BZLF1 expression by inhibiting Jun function at Zp. Consistent with this hypothesis, overexpression of ARKL1 decreased Zp activity in a Zp reporter assay, while ARKL1 silencing increased Zp activity. ARKL1 overexpression also decreased Jun activity in a Jun reporter assay. In addition, chromatin-immunoprecipitation assays confirm ARKL1 localization to Zp. The ARKL1-Jun interaction required ARKL1 sequences that we previously showed mediated binding to the CK2 kinase regulatory subunit, CK2β suggesting that CK2β might mediate the ARKL1-Jun interaction. In keeping with this model, silencing of CK2β abrogated the ability of ARKL1 to immunoprecipitate endogenous Jun and mimicked the effect of ARKL1 silencing on causing EBV reactivation. Together these findings suggest that ARKL1 binds Jun indirectly through CK2β, and that this interaction inhibits Jun activity at Zp, turning off BZLF1 expression and subsequent reactivation.

10) Defining the Contribution of TIFA to the Inflammatory Response to Adherent-invasive *Escherichia coli*

Victoria Gillmore, Scott Gray-Owen

University of Toronto

The number of people suffering from chronic diseases such as inflammatory bowel diseases, autoimmune diseases, mental disorders and cancers has increased dramatically over the last three decades. The increasing rates of these chronic systemic illnesses suggest that inflammation, caused by an aberrant immune response is unable to appropriately respond to microbial or danger signals that are new in the context of evolution. This leads to chronic inflammatory activation through continued immune cell recruitment. One such proxy for this phenome is inflammatory bowel disease whereby intestinal epithelial cells respond inappropriately to the gut microbiota leading to chronic inflammation and severe sequelae. With microbes being a necessary component for this chronic inflammation, studying the bodies response to bacterial insult is at the forefront for uncovering causes of this disease. Adherent-invasive Escherichia coli (AIEC), a unique pathovar of E. coli that has a high prevalence in ileal lesions in Crohn's disease patients compared to healthy individuals. Exposure to AIEC elicits an intense inflammatory response by intestinal epithelial cells however the cellular processes involved in this response remains to be elucidated. Similarly, the role this bacteria plays in the chronic inflammation has yet to be explained. I have shown that the recently recognized pattern recognition molecule, traf-interaction protein with forkhead associated domain (TIFA) plays an important role in this recognition, whereby TIFA-deficient cells infected with AIEC are abrogated in their cytokine response. Future work will uncover the bacterial component crucial for this aggressive inflammatory response. I hypothesize that heptose phosphates, a bacterial component recognized by the TIFA signaling axis is driving this AIEC induced inflammation in intestinal epithelial cells. This work will provide a mechanistic insight into the apparent link between AIEC and the chronic inflammatory response associated with IBD, and may assist in unraveling new therapeutic targets.

11) Epidemiology of Carbapenemase-Producing Enterobacteriaceae (CPE) in South Central Ontario

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Project Manager

Background: CPE are rapidly emerging problem worldwide. This study aims to describe the epidemiology of CPE emergence in Ontario in order to better inform CPE control programs.

Methods: The Toronto Invasive Bacterial Diseases Network (TIBDN) performs population based surveillance for CPE colonization/infection in Toronto. Microbiology laboratories report all CPE isolates to the study; annual audits are conducted. Incidence calculations use first isolates as numerator; population estimates are from Statistics Canada.

Results: CPE were first identified in 2007; incidence has increased to 1.5/100,000 (Figure 1). Bacteremia incidence has increased to 0.36 per 100,000 in 2018. Among 783 incident cases; median age is 70 (IQR 57-79yrs); 450 (57%) are male. Most common species are E coli (345,43%), K. pneumonia (391,39%) and Enterobacter spp. (79,10%); most common genes are NDM (±OXA; 432,53%); KPC (143,18%),OXA (186,23%) and VIM (34,4%). Among 345 patients only colonized when first identified 40 (12%) have subsequently had a clinical isolate (6 blood, 7 other sterile, 26 non-sterile sites). Among 646 persons with a documented history of hospitalization and travel in the year prior to identification, 199 (31%) had been hospitalized in the Indian subcontinent, 77 (12%) had been hospitalized elsewhere outside Canada, 83 (13%) had travelled to the Indian subcontinent without hospitalization, 217 (34%) had been hospitalized in Canada without hospitalization elsewhere or travel to the Indian subcontinent, 34 (5%) had not been hospitalized, but had travelled to countries outside North America and Northern Europe, and 30 (5%) had no travel or hospitalization history. Among 34 non-hospitalized, non-Indian subcontinent travelers, 7 (all OXA48) had travel to Egypt (5) or other Eastern Mediterranean countries.

Conclusion: CPE are increasing in incidence in Ontario. More than one-third of cases appear to be acquired in Canadian hospitals; travel to some non-Indian countries without hospitalization by pose an exposure risk.

12) Mucosal Immune Changes Associated with HPV Infection and High-Grade Anal Intraepithelial Neoplasia

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Introduction: Anal Human Papillomavirus (HPV) infection is common in men who have sex with men (MSM). While most HPV infections resolve spontaneously, some persist and cause anal intraepithelial neoplasia (AIN), which may in turn progress to cancer. We examined the impact of anal HPV infection and AIN on rectal immunology and surrogates of HIV transmission in MSM living with HIV.

Methods: HIV-infected, ART-treated MSM were recruited from Toronto, Canada. Anal swabs were used to 1) test for HIV RNA levels by RT-PCR and 2) screen for 46 mucosal HPV types by microsphere-based genotyping. AIN-free mucosa was biopsied, and (where applicable) additional biopsies were taken from histology-confirmed areas of AIN. Activation markers (CD38, HLA-DR), Treg markers (CD25, FoxP3) and a Th17 marker (CCR6) were assessed by flow cytometry. Statistical comparisons were assessed between groups (Mann-Whitney) and within the same individual (Wilcoxon).

Results: 46 participants were included: 16 without AIN, 14 with low-grade (LG)AIN and 16 with high-grade (HG)AIN. T cell subsets were first compared between clinically normal mucosa and AIN lesions within AIN+ individuals, and no lesion-specific alterations were apparent. When we compared clinically normal mucosa between study groups, participants with HGAIN demonstrated diffusely elevated CD38/HLA-DR co-expression on CD4+ T cells ($p= 0.035$) and an increase in Treg frequencies ($p= 0.048$). Infection with HPV16/18 was also linked to significantly higher Treg frequencies ($p= 0.021$), but Th17 frequencies did not vary with HPV status, HPV16/18 positivity or AIN stage. AIN in men with suppressed viremia was not associated with anorectal HIV shedding ($p= 0.606$)

Conclusion: HGAIN was associated with both immune activation and Treg infiltration but did not correlate with anorectal HIV shedding in ART-treated men. AIN is unlikely to contribute to HIV transmission in the context of effective ART but its effects on HIV susceptibility in HIV-negative men may merit investigation.

13) Revealing processes that confer immunity against *Neisseria meningitidis*

Elissa Currie, Scott Gray-Owen

University of Toronto

Neisseria meningitidis is a regular inhabitant of the human nasopharynx wherein it may persist asymptomatically or, under rare circumstances, invade systemically to cause sepsis and meningitis. The best way to prevent invasive disease caused by N. meningitidis is to elicit herd immunity in a population using a vaccine that can prevent both nasal colonization as well as invasive disease in vaccinated individuals. Induction of herd immunity relies on the development of mucosal immunity against N. meningitidis in the nasopharynx, however determinants of this immune response are currently unknown. To facilitate improved vaccine design, my project aims to characterize immune processes required for protection in a mouse model of nasal colonization.

Immunity to colonization can be induced in mice via repeat nasal exposure to N. meningitidis. To test the role of immune processes in mediating protection against nasal colonization, mice were depleted of target cells prior to infection. Mice depleted of either T cells or the complement system remained immune to colonization, implying that neither is an important mediator of mucosal immunity. The complement system is vital in protection against invasive meningococcal disease, and the lack of a role for it during mucosal infections was striking, emphasizing that different immune processes are required to protect different stages of N. meningitidis infection. In contrast, infection experienced mice lacking B cells or neutrophils exhibited a loss of protection, indicating both are required for mucosal immunity against N. meningitidis. Neutrophils have never been implicated in immunity against N. meningitidis, and this finding represents a novel target for vaccine development. These data shed light on the mechanism of mucosal immunity against N. meningitidis, which should facilitate development of more efficacious vaccines in the future, leading to the potential eradication of this deadly disease.

14) Investigating the role of toxin-antitoxin systems in *Legionella pneumophila* pathogenesis

Jordan Lin, Alex Ensminger

Molecular Genetics, U of T

Pathogenic bacteria must tolerate numerous stresses in order to grow and replicate, and one strategy they employ is to arrest growth until favourable conditions are restored. During its lifecycle, the bacterial pathogen Legionella pneumophila must frequently shift between states of intracellular replication and extracellular dormancy. L. pneumophila's hosts are highly variable, as it infects diverse protozoa and human alveolar macrophages, while extracellularly it occurs ubiquitously in freshwater systems. Despite the stresses of these environments, it successfully infects numerous cellular hosts and can persist in human-made water systems for prolonged periods of time, which can serve as reservoirs for recurrent disease outbreaks. Growth regulation is thus an essential component of L. pneumophila fitness, however the molecular mechanisms modulating this transition remain poorly understood.

To address this, I am examining part of the molecular circuitry governing growth regulation in L. pneumophila, namely its toxin-antitoxin systems. Toxin-antitoxin systems are typically two gene modules that encode a protein toxin capable of inhibiting growth and a corresponding antitoxin. They are nearly ubiquitous in bacterial genomes and are established regulators of growth, yet much of their function in bacterial physiology is still unknown. Due to its lifestyle, L. pneumophila is an excellent model to study the role of these systems in modulating growth to tolerate stress. Of its 7 predicted systems, I have confirmed that one encodes a functional toxin, a kinase that interacts with various components of the translational machinery. Interestingly, this locus consists of three proteins rather than the conventional two. All three proteins physically interact and likely form a complex, suggesting that this atypical architecture may represent a novel form of regulation or inhibition. Looking ahead, I plan to study the interplay between the systems in L. pneumophila to gain greater insight into their role in virulence and persistence.

15) Control of HIV infection via gene therapy with a secreted entry inhibitor

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Protein-based HIV entry inhibitors are highly effective in controlling HIV replication. Modifying patients' own cells to constitutively secrete entry inhibitors may alleviate the need for frequent injections of purified proteins and serve as an alternative to antiretroviral drug therapy. We have designed a lentiviral vector capable of secreting an entry inhibitor, soluble CD4 (sCD4), which binds to the HIV envelope proteins and inactivates virus particles. Modeling of this approach using humanized mice capable of recapitulating key aspects of the human immune system is critical to the development and implementation of this strategy. Therefore, human CD34+ hematopoietic stem progenitor cells (HSPCs) were transduced with control or sCD4 expression vectors. Transduction of HSPCs resulted in high levels of gene marking (25-30%) and expression of sCD4 (1 μg/ml). Gene-modified and unmodified HSPCs were injected into NOD/SCID/γcnull (NSG) mice. NSG hosts were capable of supporting multi-lineage differentiation from human gene-modified and unmodified CD34+ HSPCs. No major differences between lineage reconstitution by gene-modified and unmodified cells were evident. Upon challenge with HIV, humanized mice capable of secreting sCD4 demonstrated a clear reduction of HIV viral load over time compared to control humanized mice as well as higher levels of CD4+ T cells in the peripheral blood and tissues. Our work provides support for the continuous delivery of secreted entry inhibitors via gene therapy as a therapeutic alternative to antiretroviral drug therapy. We will further investigate the potential of covalently linked bi-functional fusion proteins that target multiple steps of HIV entry.

16) NaVARgator: A bioinformatics program to cluster phylogenetic trees and identify representative variants

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Phylogenetic trees are representations of the relatedness and shared ancestry of a group of variants. No matter what the variants represent – genes, proteins, genomes, species, etc – the task of clustering, or identifying variants representative of the tree arises in many different fields. Examples include selecting antigenic variants when designing a vaccine, choosing candidate strains for whole genome sequencing, or picking representative proteins for x-ray crystallography. NaVARgator performs clustering by identifying k variants as cluster centers such that the total phylogenetic distance from all variants to their nearest cluster center is minimized. The software can be run on any phylogenetic tree and allows the clustering procedure to be customized by classifying variants. If the tree contains an outgroup, or other variants to be removed from the clustering procedure, they can be assigned as “ignored”. If there are variants that should be pre-selected as cluster centers – perhaps because they are biologically important or already well studied – they can be assigned as “chosen”. The variants that the remaining cluster centers will be chosen from should be assigned as “available”. Unassigned variants will still impact the clustering calculations but cannot be selected as cluster centers.

NaVARgator provides a rich graphical user interface designed to aid the user in evaluating a cluster configuration, as well as comparing different configurations or numbers of clusters. Clustering data can be exported in a number of ways: as a customizable image of the tree, a list of variant names in the clusters or other subsets, a list of the distance between each variant and its cluster center, or a histogram of those distances. The software is available for local installation or can be accessed as a web tool.

17) Influence of LRRK2 in *Citrobacter rodentium* colitis model.

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Variants of the leucine-rich repeat kinase 2 (LRRK2) are associated with an increased susceptibility to Parkinson disease but also Crohn's disease (CD). While much research has focused on the role of LRRK2 in the nervous system, studies also suggest it may play a role in regulating immune function. The present research is designed to develop a comprehensive understanding of the role of LRRK2 in immune system modulation, and how dysfunction of this pathway may lead to the development of Crohn's disease (CD). We found that LRRK2 KO mice have a defect in migration of neutrophils to the peritoneal cavity after injection of different microbial stimuli including LPS (TLR4 ligand). Bone marrow chimera experiments confirmed the importance of the myeloid counterpart in this phenomenon. Neutrophils from LRRK2 KO mice were compromised in their ability to transmigrate in vitro in a transwell assay using fMLP as a chemoattractant. Chemotaxis was also compromised. Neutrophils from LRRK2 KO mice had lower capacity of induce actin polarization after fMLP activation in a Zigmond chamber. In parallel, we designed experiments to examine reactive oxygen species (ROS) produced in response to infection of myeloid cells with bacteria. Neutrophils from LRRK2 KO mice infected with Listeria monocytogenes were less able to restrict bacteria growth compared to WT cells. Consistent with these findings, cells from LRRK2 KO mice produced lower levels of ROS following bacterial infection. In order to determine whether myeloid cell migration is compromised in vivo during inflammation, we performed experiments in WT and KI littermates mice looking Citrobacter rodentium colitis. In this model the LRRK2 KI mice showed a faster recovery then the WT mice. With this work we aim to characterize the role of LRRK2 in intestinal homeostasis and the mucosal barrier maintenance, including how its deficiency may predispose an individual to developing CD.

18) Controlling HIV-1 replication by small molecule activation of Raf-MEK1/2-ERK1/2 signaling through G protein-coupled receptors

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The ability of HIV-1 to evolve resistance to existing antiretroviral drugs has stimulated research into alternative means of controlling this infection. We assayed >60 modulators of RNA processing and identified compound 191 as a potent inhibitor of both wild-type (IIIB, nL4-3, and N54) and drug-resistant strains of HIV-1 in CD4+ T cells (IC50: ~700 nM), LAI in HeLa cells (IC50: 750), and BaL in CD4+ primary T cells (IC50: 1.8 μM) with limited effects on cell viability. 191 dramatically reduces expression of four essential HIV-1 structural (Gag/Env) and regulatory (Tat/Rev) protein/polyproteins. These results are associated with altered viral RNA accumulation and transport: reducing levels of HIV-1 unspliced and singly-spliced RNAs (oversplicing) and Rev, respectively. Consistent with inducing oversplicing of HIV-1 RNAs, 191 causes a 2-fold increase in abundance (and modification) of serine/arginine-rich splicing factor 4 (SRSF4) and enhances the activity of nearly half of all SR proteins assayed (SRSF1/3/4/9 and Tra2α) for inducing alternative splicing. In contrast, this compound causes a 2-fold decrease in accumulation of SRSF1 and reduces the function of SRSF10 and SRSF7 whereas it elicits limited change on the abundance of SRSF2/3/6/7/9 and Tra2β and activity of SRSF2 and Tra2β. Decreased accumulation of Tat was reversible by addition of MG-132, suggesting that 191 enhances degradation of this viral factor. Conversely, 191 causes minimal perturbation in alternative splicing (0.25% by ≥ 20%) and expression of host RNAs (0.01% by ≥ 5 fold) as well as synthesis and abundance of host proteins (0.02% by ≥ 2 fold). Inhibition of HIV-1 gene expression by this compound requires G protein-coupled receptor signaling of Raf-MEK1/2-ERK1/2. Supporting this hypothesis, overexpression of variants of a small G protein suppressed HIV-1 gene expression. These findings reveal the potential of targeting a host intracellular signaling pathway as a new alternative approach for controlling HIV-1 infection.

19) A mitochondria-derived metabolite restricts influenza virus infection by disrupting the viral replication cycle

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Influenza A virus (IAV) is the etiological agent of a highly contagious acute respiratory disease, which causes a considerable socioeconomic burden despite annual vaccination campaigns. Therefore, it is essential to better understand IAV-host cells interaction to help design innovative antiviral therapies. In that regard, recent studies revealed the interplay between metabolic and immune signaling pathways. However, it remains unknown whether IAV alters lung tissues metabolism and what is its potential functional consequence. Using in vitro and in vivo models as well as human respiratory fluids and in-depth metabolomics analysis, we first found that IAV infection alters the glycolysis and mitochondrial oxidative respiration in lung tissues, leading to the accumulation of several immunometabolites in the bronchoalveolar airspaces. We next focused on one mitochondria-derived metabolite (coined here "MDA") as its accumulation was found not only in the lungs of IAV-challenged mice but also in the tracheal fluids of IAV-infected patients. Remarkably, we found that MDA exhibits a potent antiviral activity both in vitro and in vivo as it inhibits H1N1 and H3N2 IAV strains and it strongly decreases IAV-triggered inflammatory response. The underlying inhibiting mechanism involves a disruption of IAV replication cycle as MDA prevents specifically the nuclear export of the viral proteins NP and NS1. Finally, we showed that mice receiving MDA through the intranasal route are more resistant to IAV pneumonia than mock-treated animals. Hence, our study identifies the metabolite MDA as a novel component of the host antiviral arsenal.

20) How adaptive immunity constrains the composition and fate of large bacterial populations

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Department of Physics

Features of the CRISPR-Cas system suggest that its effect is not limited to individual bacteria but may control the fate and structure of whole populations. Emphasizing the population-level impact of the CRISPR-Cas system, recent experiments show that some bacteria regulate CRISPR-associated genes via the quorum sensing pathway. Here we present a two-part model of interacting bacteria and phages to explore both the effects of stochasticity and co-evolution on the population as a whole.

Even with limited phage diversity, spacer abundance is highly stochastic and variable. In spite of this, the population-level rank-abundance distribution of spacers is time invariant, a surprising prediction that we test with dynamic spacer-tracking data from literature. This distribution depends on the state of the competing phage–bacteria population, which due to quorum-sensing-based regulation may coexist in multiple stable states.

Adding phage mutations in our model, we track the co-evolution of the bacteria and phage populations and find that the overall population reaches a stable state of constant “motion” through sequence space: phages continually mutate to new sequences, and bacteria follow with a constant lag that depends on the effectiveness of the CRISPR system. Counterintuitively, the lag is larger when the CRISPR system is more effective.

This study lays out a path toward a phenomenological framework for understanding microbial dynamics and may provide insights into complex and diverse natural populations where microscopic modeling is plagued by overparameterization and overfitting.

21) Successes of anti-RSV prophylaxis among infants in Ontario: results from a multi-decade, population-based controlled interrupted time series analysis using health administrative data

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Background: Respiratory syncytial virus (RSV) is the leading cause of lower respiratory infections and hospitalization among children worldwide. While there are yet no licensed vaccines, RSV prophylaxis (Palivizumab) is available to children at increased risk of severe RSV-related illness. In Ontario, Palivizumab has been publicly funded for high-risk infants since 2002 and through a national special access program since 1998.

Objective: to evaluate changes in severe pediatric RSV-related illness (admissions, deaths) following the introduction of Palivizumab in Ontario. We further investigated potential differences across socio-economic factors.

Methods: All infants born in Ontario Jan 1st, 1993 through Dec 31st, 2016 were followed until the earliest of: 2nd birthday; death; moving out of province; or end of follow-up, June 30th, 2017. All hospital admissions for bronchiolitis, RSV pneumonia, or flagged with an RSV diagnosis and all RSV-related deaths were identified using linked administrative health data. High-risk infants were defined according to program guidelines identifiable through administrative health data. Interrupted time series analyses were conducted to assess changes in pre- vs. post-program rates and trends. Differences across neighbourhood income quintiles, rurality, and maternal age were also investigated. All analyses were stratified by age at infection (<6, 6-12, 12-24 months).

Results: Over 3 million infants and nearly 87,000 admissions were identified. RSV burden was substantially greater among infants <6 months. Statistically significant declines were seen among all <6-month olds following both programs (1998: $p=0.0167$, 2002: $p<0.0001$). Rates markedly dropped among high-risk infants over time (60%). Even within high-risk infants, rates were consistently greater among children from low income areas and born to teenage mothers; however, these gaps substantially narrowed over time.

Conclusions: This large, population-based study of infants spanning 24-years found significant declines in severe RSV-related illness (hospital admissions, deaths) following the introduction of palivizumab in Ontario, along with evidence to suggest narrowing social inequities.

22) Examining the Molecular Basis of Heme Acquisition in *Acinetobacter baumannii*

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Multidrug resistant A. baumannii is an urgent public health threat, and has recently topped the World Health Organization's list of deadly superbugs that require immediate research into the development of novel therapeutics. One potential weakness is that A. baumannii needs to acquire essential metals like iron for its growth and survival during infections. Heme is the largest source of iron in humans, but it is found in the blood tightly bound to large bulky proteins such as hemoglobin and albumin. Our investigation into the A. baumannii protein machinery required for heme acquisition began with a previous bioinformatics study which identified a potential gene cluster involved in heme utilization, encoding a predicted TonB Dependent Receptor called TBDR2, a beta-barrel protein known to transport limiting nutrients like heme across the outer membrane. Closer inspection of this cluster led us to discover that two adjacent genes were misannotated, and are predicted to encode a surface lipoprotein (SLP) and surface lipoprotein assembly modulator (Slam). Our lab had previously shown that Slam proteins function as conduits for the translocation of SLPs to the cell surface, and that a Slam deletion renders a human pathogen avirulent likely due to the absence of SLPs that normally play key roles as virulence factors. To date, I have shown that AbSLP is a cell surface heme receptor, dependent on Slam for its surface localization. Furthermore, TBDR2, AbSLP and AbSlam are required for growth on sub-nanomolar concentrations of human hemoglobin and heme bound to serum albumin. We think that AbSLP and TBDR2 work together as a two component or bipartite receptor for pirating heme and importing it into the bacterial cell. Our characterization of this heme uptake system sheds light on an unexplored area of A. baumannii biology and has revealed potential therapeutic targets that may be exploited in the future for specific antibiotics or vaccines.

23) Dual RNA-Seq characterization of host and pathogen gene expression in liver cells infected with Crimean-Congo Hemorrhagic Fever Virus

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Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus that can cause a hemorrhagic fever in humans, with a case fatality rate of up to 40%. Cases of CCHFV have been reported in Africa, Asia, and southern Europe; and recently, due to the expanding range of its vector, autochthonous cases have been reported in Spain. Although it was discovered over 70 years ago, our understanding of the pathogenesis of this virus remains limited.

We used RNA-Seq in two human liver cell lines (HepG2 and Huh7) infected with CCHFV (strain Ib 10200), to examine kinetic changes in host expression and viral replication simultaneously at 1 and 3 days post infection. Through this, numerous host pathways were identified that were modulated by the virus including: antiviral response and endothelial cell leakage. Notably, the genes encoding DDX60, a cytosolic component of the RIG-I signalling pathway and OAS2 were both shown to be dysregulated. Interestingly, PTPRR was induced in Huh7 cells. This has been associated with the TLR9 signalling cascade, and polymorphisms in TLR9 have been associated with poor outcomes in patients. Additionally, we performed whole-genome sequencing on CCHFV to assess viral diversity over time, and its relationship to the host response. As a result, we have demonstrated that through next-generation mRNA deep-sequencing it is possible to not only examine mRNA gene expression, but also to examine viral evolution. This demonstrates a proof-of-principle that CCHFV specimens can be analyzed to identify both the virus, and host biomarkers that may have implications for prognosis.

24) Environmental sampling for the surveillance of influenza A virus in swine

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The potential for genetic reassortment and generation of novel strains of influenza A virus in swine has significant implications for public health. Enhanced surveillance methods through environmental sampling can be a beneficial tool to detect and monitor influenza A virus activity. The objective of this study was to detect influenza A in environmental samples collected from a farrow-to-feeder facility swine operation, and determine whether viral RNA sequence data can be derived from these samples. Sampling was conducted between May 2018 to February 2019. Pooled oral fluids and surface swabs were collected from each pen in three rooms. In addition, room-level air samples were obtained using two low (the polytetrafluorethylene (PTFE) sampler and the National Institute for Occupational Safety and Health (NIOSH) 2-stage cyclone sampler) and two high (the Coriolis cyclonic sampler and the Smart Air Sampler System (SASS) 3100. volume bioaerosol samplers. Samples were analyzed by quantitative RT-PCR. Samples with a CT value < 36 were deemed positive and quantifiable, and samples with a CT value between 36 to 38 were considered suspect positive. Eighty- eight oral fluid samples, 34 surface swabs, and 198 bioaerosol samples were collected. In 75 rooms deemed positive by oral fluids, 23.8%, 21.4%, 35%, and 47.1% of samples were positive by PTFE, coriolis, NIOSH, and swabs, respectively. Suspect positive samples were detected in 23.8%, 64.3%, 45%, and 23.5% by PTFE, Coriolis, NIOSH, and swabs, respectively. Of 23 oral fluids and 9 PTFE filters sequenced, whole genomes were sequenced in 13 and 1 samples respectively, and partial genomes from 10 and 8 oral fluid and bioaerosols samples respectively. Environmental sampling is an effective approach for surveillance of influenza A however, bioaerosol sampling and sample processing requires further optimization to enhance sequencing yield.

25) An Update on the Role of Imaging in the Care of Patients with Schistosomiasis

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"Schistosomiasis leads to significant morbidity and mortality worldwide. Infection with Schistosoma mansoni and S. japonicum can lead to severe hepatic disease including periportal liver fibrosis and portal hypertension. Previous studies recommend the use of abdominal imaging to detect early hepatic changes and improve disease outcome. However, there are no recently published or authoritative resources to guide the use of imaging in the initial diagnosis of schistosomiasis. We searched available literature regarding the role of imaging in the evaluation of patients with schistosomiasis and aim to synthesize clinical recommendations. Eight electronic databases were searched: Ovid Medline, EMBASE, Cochrane Library of Systematic Reviews, Epistemonikos, Global Health, NICE, TRIP and LILACS with the following search terms: [Schistosomiasis OR (Schisto* AND (mansoni OR japonicum))] AND [CT OR (computed AND tomography) OR Ultraso* OR Sonogr* OR MRI OR (Magnetic AND resonance AND Imaging) OR Echo OR Imaging] AND [Liver OR periportal OR peri-portal OR fibrosis OR hepat* OR echogenic* OR (portal AND hypertension)] from database inception to February 28, 2019. A total of 2977 articles were identified: 691 articles on Ovid Medline, 30 Cochrane, 1035 Embase, 10 Epistemonikos, 516 Global Health, 34 NICE, 529 TRIP, and 132 LILACS. A total of 1933 articles remained for title screening after de-duplication. Titles, abstracts and full-texts were systematically double-screened by two reviewers and a tertiary arbitrator. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was employed. Two reviewers performed data extraction and quality of the studies was assessed with the Grading of Recommendations Assessment, Development and Evaluation (GRADE). Data were summarized using qualitative and quantitative measures to evaluate the role of imaging in the clinical management of schistosomiasis. Synthesizing the current literature on abdominal imaging in the evaluation of schistosomiasis can translate into clinical recommendations for improved risk stratification and overall management of schistosomiasis.

26) Low Sequence Heterogeneity of *Plasmodium falciparum* Isolates Imported to Ontario, Canada from West Africa over a 10-year Period with Increased Molecular Markers of Resistance to Proguanil

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Approximately 200 cases of malaria are imported to the province of Ontario annually, with the majority due to Plasmodium falciparum (Pf) originating from West Africa. We performed sequence analyses of Pf isolates returning from Ghana and Cameroon over a 10-year period to understand patterns of genetic heterogeneity and molecular drug resistance markers over time. We identified 36 Pf isolates from Ghana (18 from 2006-2008 and 18 from 2014-2016); and 16 from Cameroon throughout 2006-2016. DNA was extracted and regions commonly used for strain typing were analyzed including: merozoite surface potein (msp) 1 and 2; erythrocyte binding antigen (eba) 175; and glutamate-richprotein (glurp) regions. Molecular resistance markers including: cytochrome B (cytB) and dihydrofolate reductase (dhfr) for resistance to atovaquone-proguanil (Malarone®); atpase6 and kelch13 for artemisinin and derivatives; and chloroquine resistance transporter (Pfcrt) for chloroquine were analyzed. Phylogenetic tree analysis revealed some sequence heterogeneity within Ghanian and Cameroonian isolates, however, there was no clustering of isolates over time. All isolates were wild type on cytB codon 268. Isolates from Cameroon all had triple codon 51, 59, and 108 mutations at dhfr conferring resistance to proguanil, whereas isolates from Ghana had an increase of such mutations from 39% (7/18) in 2006-2008 to 83% (15/18) in 2014-2016 ($p=0.0153$). Eight percent (3/36) of Ghanian isolates had a mutation in codon 623 of atpase6, while all Cameroonian isolates were wild type. No mutations were observed at atpase6 codon 769 or kelch13 codons >440. In Pfcrt codon 76, 27% (7/26) of Ghanian isolates were mutant compared to 50% (6/12) of those from Cameroon. Pf isolates from Ghana demonstrated increasing molecular markers of resistance to proguanil, but remain wild type to the partner drug atovaquone in Malarone. The relatively high percentage of molecular mutants to chloroquine resistance still predominates throughout West Africa. The low sequence heterogeneity suggest there was no major evolutionary genetic changes over the years.

40) Management of Common Intestinal Parasites in Pregnancy: A Systematic Review of Maternal Outcomes

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Parasitic infections in pregnancy necessitate consideration of numerous factors including the potential safety, efficacy, and tolerability of antiparasitic drugs for the mother and potential maternal-to-child parasite transmission risk during pregnancy and delivery. For these considerations, a substantial knowledge gap exists, with no definitive published and authoritative resource to guide clinical decision-making. We aim to map the available literature regarding the efficacy, safety, and tolerability of treatment of intestinal parasites in pregnancy, and synthesize the available literature on specific parasitic infections and anti-parasitic agents. Five electronic databases were searched (Medline,EMBASE, CINAHL, Cochrane Library of Systematic Reviews, and CENTRAL) and titles, abstracts, and full-texts of included studies and reviews were screened from database inception to July 2018, without language restriction. Two independent reviewers with a tertiary arbitrator screened all systematic reviews, randomized controlled trials, cohort studies, smaller observational studies, case-control studies, case series, and case reports assessing or reporting the efficacy, safety, or tolerability of anti-parasitic drugs used in management of parasitic infections during pregnancy. Two independent reviewers extracted the data and assessed trial quality using the GRADE approach. Data were summarized using qualitative and quantitative measures for specific parasitic infections as well as efficacy and safety of anti-parasitic agents. Risk of bias for each study was determined. Preliminary data showed Mebendazole decreased the prevalence of soil-transmitted helminth infection in pregnant mothers. With increased international travel and migration of migrant and vulnerable populations, it can be expected that health practitioners will be faced with managing parasitic infections in pregnant patients. Currently, quality evidence supporting specific management strategies is limited. Synthesizing the current literature on anti-parasitic agents and treating parasitic infections in pregnancy can translate into multidisciplinary clinical recommendations for improved pregnancy care.

41) Management of Common Intestinal Parasites in Pregnancy: A Systematic Review of Fetal and Neonatal Outcomes

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Parasitic infections in pregnancy necessitate consideration of numerous factors including potential maternal-to-child parasite transmission risk during pregnancy and delivery and potential anti-parasitic drug toxicity to the fetus and development of the newborn. For these considerations, a substantial knowledge gap exists, with no definitive published and authoritative resource to guide clinical decision-making. We aim to map the available literature regarding the efficacy, safety, and tolerability of treatment of intestinal parasites in pregnancy, and synthesize the available literature on specific parasitic infections and anti-parasitic agents. Five electronic databases were searched (Medline,EMBASE, CINAHL, Cochrane Library of Systematic Reviews, and CENTRAL) and titles, abstracts, and full-texts of included studies and reviews were screened from database inception to July 2018, without language restriction. 2479 articles were identified and 1774 articles were retrieved for title, abstract and full-text screening. Two independent reviewers with a tertiary arbitrator screened all systematic reviews, randomized controlled trials, cohort studies, smaller observational studies, case-control studies, case series, and case reports assessing or reporting the efficacy, safety, or tolerability of anti-parasitic drugs used in management of parasitic infections during pregnancy. Two independent reviewers extracted the data and assessed trial quality using the GRADE approach. Data were summarized using qualitative and quantitative measures for specific parasitic infections as well as efficacy and safety of anti-parasitic agents. Risk of bias for each study was determined. Preliminary data showed Mebendazole treatment in mothers with soil-transmitted helminth infection had no adverse birth outcomes and may have a protective effect against “very low birth weight” newborns. With increased international travel and migration of migrant and vulnerable populations, it can be expected that health practitioners will be faced with managing parasitic infections in pregnant patients. Currently, quality evidence supporting specific management strategies is limited. Synthesizing the current literature on anti-parasitic agents and treating parasitic infections in pregnancy can translate into multidisciplinary clinical recommendations for improved pregnancy care.

42) Validation of a Multiplex Real-time PCR Gastrointestinal Helminth Panel

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Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens, however, it requires high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We validated a commercial multiplex parasitic real time PCR panel detecting 7 helminths: Ascaris spp. (As), Enterobius vermicularis (Ev), Hymenolepis spp. (Hy), Necator americanus (Na), Strongyloides spp. (St), Taenia spp. (Ta) and Trichuris trichiura (Tt) at Public Health Ontario, Canada. We analyzed 86 banked frozen fecal specimens including: 86 specimens without any pre-treatment and 86 specimens pre-treated with ASL buffer, containing As (n=23), Ev (n=13), Hy (n=1), Ta (n=4), St (n=33), Tt (n=10), and 3 mixed infections. A panel of protozoa and helminth specimens not covered in these assays was used for cross reactivity evaluation. DNA extraction and PCR were conducted with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms. Where fully evaluable due to sufficient numbers, sensitivity, specificity, positive predictive-, and negative predictive values without pre-treatment were: 48%, 100%, 100% and 84% for As; 77%, 100%, 100% and 96% for Ev; 57%, 98%, 95% and 77% for St; and 100%, at all metrics for Hy and Ta; and with ASL pre-treatment were: 65%, 100%, 100% and 89% for As; 100% at all metrics for Hy and Ta; and 53%, 100%, 100% and 75% for St. No cross-reactivity was observed with other protozoa or helminths. The platform had high sensitivity for detection of a small number of Ta and Hy, but suboptimal sensitivity for Ev and St. Further validation with greater numbers of specimens is required for performance determination with other helminths and those without sufficient numbers to report in this analysis.

43) Validation Of A Multiplex Real-time PCR Gastrointestinal Parasites Panel

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Microscopy is the conventional method for identification of gastrointestinal parasitic pathogens in fecal samples, however, it presents numerous challenges including high technical expertise and prolonged turnaround time. Molecular methods provide higher throughput and potentially higher sensitivity and specificity. We sought to validate a commercial multiplex parasitic real time PCR panel detecting 6 protozoal pathogens: Blastocystis hominis (Bh), Cryptosporidium, Cyclospora, Dientamoeba fragilis (Df), Entamoeba histolytica (Eh) and Giardia lamblia (Gl) in unpreserved fecal specimens submitted for diagnostic parasitology. We analyzed 192 specimens, including 84 banked, frozen known positive specimens containing all of the targeted pathogens (8 Bh, 13 Crytosporidium, 13 Cyclospora, 10 Df, 15 Eh, 13 Gl and 12 mixed protozoal infections) and 108 fresh specimens randomly selected from our prospective parasitology submissions, including 4 Bh, 3 Df, 2 mixed infections, and 99 microscopy negatives. DNA extraction and PCR were setup with the Hamilton Starlet automated platform and Seegene's extraction and PCR kits. Microscopy was the reference standard for all organisms with stool ELISA as an additional reference assay for Eh. Sensitivity, specificity, positive predictive and negative predictive values of the enteric multiplex were: 96%, 90%, 60%, and 99% for Bh; 100% for Cryptosporidium; 79%, 100%, 100%, and 98% for Cyclospora; 86%, 86%, 86%, and 98% for Df; 81%, 100%, 100%, and 98% for Eh; and, finally, 94%, 85%, 85% and 99% for Gl, respectively. The platform had high sensitivity for Bh, Cryptosporidium and Gl, but suboptimal sensitivity for detection of Cyclospora, Df, and Eh. Low positive predictive value for Bh may reflect challenges to accurate microscopic identification of this organism. Negative predictive value was excellent for all targets, supporting that the platform accurately determines true negatives. Limit of detection was as follows: 8 parasites/g stool for Bh; 9 parasites/g stool for Cryptosporidium; 38 parasites/g stool for Cyclospora; 697 parasites/g stool for Df; 47 parasites/g stool for Eh; and 22 parasites/g stool for Gl. This particular enteric multiplex platform provides a useful diagnostic tool for Bh, Cryptosporidium, and Gl. Further optimization of the assay is required for Cyclospora, Df, and Eh prior to clinical use.

44) Accuracy of Diagnostics in Tegumentary Leishmaniasis: A Systematic Review

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Tegumentary leishmaniasis (TL) is characterized by cutaneous and mucocutaneous ulcerative skin lesions, caused by Leishmania parasites, that can potentially disfigure the midface. The clinical presentation of TL is similar to that of epidemiologically overlapping fungal and mycobacterial infections, thereby necessitating confirmatory diagnostics to inform appropriate treatment. Laboratory diagnostic techniques for TL include the leishmanin skin test; microscopic identification of amastigotes from skin aspirates, biopsies and scrapings; culture; and molecular assays. We aim to determine optimal methods to accurately and efficiently diagnose TL to improve diagnostic stewardship. We searched five databases from inception to July 16, 2018 including Ovid MEDLINE, Embase, LILACS, Cochrane Library and Scopus with the following search terms: (""cut* leish*"" OR ""muc* leish*"" OR ""teg* leish*"") AND (diagnosis OR diagnostic accuracy OR sensitivity OR specificity OR stard OR test*) AND NOT (viscer*). All systematic reviews, diagnostic trials and observational studies were included. Titles, abstracts and full-texts are systematically doubled screened by two reviewers with a tertiary arbitrator. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Quality Assessment of Diagnostic Accuracy Studies (QUADAS) will be employed. 6745 papers were identified from the five databases and 1278 papers remained for abstract evaluation (3391 removed) after title screening, where non-human, non-TL, non-diagnostic and case report articles were excluded. Abstract and full-text screening will be conducted. Data will be extracted from full-texts and assessed using QUADAS for selection and information bias. Heterogeneity of the studies will be determined and meta-analysis performed as appropriate. TL cannot be distinguished from competing infectious etiologies clinically, thus necessitating confirmatory diagnostics. A knowledge synthesis of accurate diagnostic assays can provide insight into the optimal approach for TL confirmation and subsequently guide therapy.

45) Prevention of Hepatitis B Virus Transmission in the Hemodialysis Setting: Is Isolation Necessary?

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Background

The CDC recommends isolation of Hepatitis B Surface Antigen-positive (HBsAg+) patients with dedicated rooms, dialysis machines, and staff. Since this recommendation was released in 2001, our institution has not adopted this practice due to limited isolation capacity. Our institution does follow other CDC recommendations, including serologic surveillance, vaccination of susceptible host, disinfection of dialysis machines, single vial use, aseptic technique, and routine common area surface cleaning. It is unknown whether the risk of HBV transmission is increased when HBsAg positive dialysis patients are not isolated.

Methods

We retrospectively reviewed patients receiving hemodialysis at a single institution in Toronto, Canada between 2002 and 2017 who underwent surveillance HBV testing. HBV acquisition was defined as acquiring at least one of 1) newly positive HBsAg, 2) HBV DNA, 3) Hepatitis B Core Antibody (HBcAb) > 6 months after initiation of hemodialysis at our center; and having epidemiological linkage to a known infected patient. Epidemiological links were determined based on temporal and geographic proximity to HBV-positive patients. Exposure was defined as use of the same dialysis machine as an HBV-positive patient within 7 days, or receiving dialysis directly adjacent to an HBV-positive patient.

Results

12 out of 1448 patients had a change in serology during the study period after initiating dialysis. However, based on epidemiological investigations, none of the conversions in HBV serology were linked to exposure to an HBV-positive patient in the dialysis unit. Furthermore, none of these 12 patients developed clinically significant hepatic disease.

Conclusion

Our study challenges the current CDC recommended practice of isolating HBV-positive patients in the hemodialysis setting. Routine hand hygiene, internal and external disinfection of hemodialysis machines between patients, single vial use, and routine environmental cleaning may be sufficient at preventing transmission.

46) Analysis of influenza virus whole genomes of swine and avian origin in Canada

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From a public health perspective, surveillance of influenza viral genomes in Canada is critical to providing an effective response strategy against emerging strains of pandemic potential. This project investigates the genomic content and phylogenetic relationship of influenza virus strains of swine and avian origin in Canada to detect pathogenic polymorphisms and potential re-assortment events. Using novel bioinformatic tools from the Influenza Research Database, the whole genomes of viral strains were analyzed for the presence of single nucleotide polymorphisms (SNPs) associated with pathogenicity. Phylogenetic trees were constructed to investigate whether these SNPs originated in one population as a result of a re-assortment event. In total, 421 influenza viruses of swine origin and 830 influenza viruses of avian origin from 9 provinces over 40 years were available in the Influenza Research Database and analyzed. This study primarily focuses on the pathogenic E627K mutation in the PB2 segment but point mutations from other segments were also investigated. It was found that the segment containing the E627K mutation may have been introduced to the swine influenza virus population as a result of a re-assortment event with a strain from the avian influenza viral population. Significant geographic and temporal gaps in sequence availability were noted, with limited overlap between sequence data originating from swine and avian sources. Further studies are required to elucidate the origins of this important mutation and enhanced surveillance efforts must be made to generate fulsome and actionable data.

47) Hematologic Parameters of Acute Dengue Fever versus Other Febrile Illnesses in Ambulatory Returned Travelers

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Dengue fever is a mosquito-borne acute febrile illness, which is acquired from the tropics and subtropics. Recent years have seen dramatic rises in the burden of dengue worldwide, including in travelers. Thrombocytopenia and lymphopenia are common hallmarks of dengue, however neutropenia is a prominent, yet less frequently reported trend. The importance of utilizing abnormal blood count findings for guiding the early diagnosis and treatment of dengue will be explored in this paper. A retrospective case control study was undertaken on data from February 2014 to December 2017. Patients presenting through the Rapid Assessment of Febrile Travelers program with dengue were compared to those presenting with other febrile illnesses (OFI). Patient demographic, day of illness, and available neutrophil, lymphocyte and platelet counts from day 1-14 of illness were collected. Analyses were stratified by day of illness. 18 patients were included in the dengue group and 151 were in the OFI group. Thrombocytopenia within the dengue cohort was found to be significantly greater than the OFI group (77.8% vs 23.2%, $p < 0.0001$). Neutropenia was also significantly more common in dengue patients than in those with OFI ($p < 0.0001$), with 72.2% vs. 12.6% of patients, respectively, demonstrating neutropenia during their illness. There was also a significant difference in the frequency of lymphopenia between the dengue and OFI study groups, 88.9% vs. 35.8% respectively ($p<0.0001$). When grouped by day of illness, significantly lower mean platelet and neutrophil counts were evident in patients infected with dengue compared to the OFI group ($p<0.0001$). In patients with a relevant travel history, neutropenia and thrombocytopenia should help guide provisional diagnosis of acute dengue infection. As advanced diagnostic testing is often inaccurate or delayed by prolonged turn-around times, these simple laboratory features can guide the early care and follow-up of febrile returned travelers with suspected dengue infection.

48) A Systematic Review of Scorpion Envenomation Therapeutics and Antivenom Accessibility

Avinash N. Mukkala, Christian Lecce, Aisha Khatib, Michael A. Klowak, Priyanka Challa, Eric Shao, Jason Kwan, Tianna Chong-Kit, Jamie Sookhoo, Emma Hagopian, Dylan Kain, Mofe Adeosun, Andrea K. Boggild

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Scorpions (Scorpiones) are eight-legged arthropods of the class Arachnida. With increased human migration and transcontinental shipment of produce from the tropics, the incidence of scorpion envenomations may increase in non-endemic areas. We aim to synthesize existing evidence around prevention and treatment of scorpion envenomations into a clinical resource, including provision of information on access to, and indications for, antivenom usage. PubMed (NCBI), MEDLINE (OVID), EMBASE (OVID), Cochrane Database of Systematic Reviews (CIDR) and TOXLINE (TOXNET) were searched from inception to June 2018 using combinations of the search terms ""scorpion"" and ""envenomation". Iterative inclusion and exclusion of search terms was employed to maximize article extraction. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. Evidence will be summarized using descriptive measures for each intervention type, as well as a qualitative synthesis. Meta-analysis will be planned if sufficient efficacy measures exist. 961 MEDLINE articles, 1053 PubMed, 1486 EMBASE, 0 CIDR and 149 TOXLINE records were retrieved for title and abstract screening; after a multi-step deduplication pipeline, 1928 remained. After title and abstract screening, 422 studies were eligible for inclusion. Some of the main medically important species include: Mesobuthus tamulus, Androctonus australis, Hemiscorpius lepturus, Tityus serrulatus, and Centruroides sculpturatus. Data will be grouped and summarized for ease of clinician use by prevention, therapeutic strategies, geographic location and species. The recommended mode of treatment and management will be provided on an evidence-based, per-species basis. Increased transcontinental movement of people and tropical produce has facilitated importation of scorpions to non-endemic regions where clinicians lack familiarity with envenomation syndromes and appropriate therapeutics. Synthesizing the current evidence around therapeutic strategies for scorpion envenomations can inform the development of appropriate treatment and prevention protocols.

49) A Systematic Review of Virulence Factors in the Leishmania Genus

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Parasite-determined factors play a complementary role in the pathogenesis of leishmaniasis, a disease caused by protozoans of the genus Leishmania with diverse and species-specific clinical manifestations. Virulence factors (VFs), or pathogen moieties facilitating disease, can potentiate host cell damage by Leishmania species via increased expression, host cell invasion, stress tolerance, and modulation of the host immune system. Due to large eukaryotic genomes in Leishmania species, there is a wide array of VFs which contribute to different aspects of pathogenesis. Here we conduct a comprehensive, systematized review of the literature around VFs in Leishmania spp. and construct a complete picture of parasite-determined contributors to the pathogenesis of various clinical forms of leishmaniasis. PubMed (NCBI), MEDLINE (OVID), EMBASE (OVID), Web of Science, and LILACS (VHL) were searched from inception to July 2018 using combinations of the search terms ""virulence factor*"", ""Leishmania"", and ""Leishmaniasis*"", while accounting for unique database syntax. Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. For the systematic review, we will include primarily molecular and mechanistic pathogenesis studies in various model systems, observational studies, review studies, cohort studies, as well as clinical trials. Of 2620 articles remaining after title and abstract screening, some major VFs identified in the Leishmania genus are: heat shock proteins (HSP23, HSP70), cysteine peptidases (CPB), mannose phosphate isomerases (MPI), metalloproteases (GP63), and elongation factors (EF1-alpha), among many others. Data will be grouped and summarized by species, geographic region of endemicity, and VFs. This systematic compilation of mechanistic VF data will add to the large body of work in molecular pathogenesis of kinetoplastids and enhance our understanding of species and regional variations in Leishmania pathogenesis.

50) Spider Envenomations Therapeutics and Antivenom Accessibility: A Systematic Review

Christian Lecce, Avinash N. Mukkala, Aisha Khatib, Michael A. Klowak, Pryanka Challa, Eric Shao, Jason Kwan, Tianna Chong-Kit, Jamie Sookhoo, Emma Hagopian, Dylan Kain, Mofe Adeosun, Andrea K. Boggild

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Spiders are a group of arthropods in the order Araneae and class Arachnida which have eight legs and fangs. Modern advancements in transportation allow increased human travel to areas which are endemic to spiders, increasing the possibility of envenomation. Physicians could select the optimal envenomation treatment using a clinical resource that compares efficacy statistics of antivenom versus other therapeutics. Our goal is to compile existing prevention and treatment data in the literature in order to synthesize this clinical resource. PubMed (NCBI), MEDLINE (OVID), EMBASE (OVID), Cochrane Database of Systematic Reviews (CIDR) and TOXLINE (TOXNET) were searched from inception to June 2018 using combinations of the search terms “spider,” and ""envenomation*. Iterative inclusion and exclusion of search terms was employed to maximize extraction. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. Evidence will be summarized using descriptive measures for each intervention type, as well as a qualitative synthesis. Meta-analysis will be planned if sufficient efficacy measures exist. 961 MEDLINE articles, 1053 PubMed, 1486 EMBASE, 0 CIDR and 149 TOXLINE records were retrieved for title and abstract screening; after a multi-step de-duplication pipeline, 1928 remained. Following abstract screening, 282 full-text records were eligible for inclusion. Upon initial review of these records, Latrodectus hasseltii, Latrodectus mactans, Loxosceles reclusa, and Phoneutria spp. were the most medically relevant. Data will be grouped and summarized by prevention, therapeutic strategies, geographic location and species. The recommended mode of treatment and management will be provided on an evidence-based, per-species basis. Increased transcontinental movement of people and tropical produce has facilitated importation of arachnids to non-endemic regions where clinicians lack familiarity with envenomation syndromes and appropriate therapeutics. Synthesizing the current evidence around therapeutic strategies can inform the development of treatment and prevention protocols.

51) Nutriome Effects on Host Immunological Control of Protozoal Infections

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Immunologic control of parasitic infections is a combination of humoral and cellular immunity, where genetic factors including host nutritional status underline the host immune response to protozoal infections. Inadequate nutritional status impairs the functioning of the immune system, resulting in increased susceptibility to protozoal infections. We aim to synthesize such knowledge, focusing on the interrelationships between nutrients and immune function. More specifically, we will demonstrate the ways in which nutrient deficiencies such as zinc, iron and vitamin A impact immune response and defence in patients with infectious diseases such as Malaria. Five electronic databases were searched including PubMed, Embase, Medline, Scopus, and LILACS with combinations of search terms such as Parasite* AND (Immunology OR Immunity OR Immune System OR Immune Function OR Immune Impairment OR Immune Response OR Immune Status) from database inception to March 13, 2019. A total of 30 872 articles were retrieved: 15 254 articles on PubMed, 8192 on Embase, 5909 on Medline, 1411 on Scopus, and 106 on LILACS. After eliminating duplicates using Mendeley software, a total of 21 821 articles remained for title screening. Titles, abstracts, and full-text articles will be systematically double screened by two reviewers with a tertiary arbitrator. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) will be implemented. Data extraction will be performed by two reviewers and the quality of the articles will be critically evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. The data will be summarized to systematically map published literature that will illuminate a number of ways in which nutrient deficiencies alter and impair immune function in patients with protozoal infections. This synthesized body of information will ultimately inform therapeutic decisions in the context of protozoal infections and will aim to improve patient prognosis.

52) Chemical Biology Analysis of Protein Kinases in the Fungal Pathogen *Candida albicans*

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Invasive fungal disease is responsible for more than 1.5 million deaths globally each year. Candida albicans is a primary causal agent of serious mycotic infection with mortality rates of ~40% despite therapeutic intervention. Poor patient outcomes are a result of the limited number of antifungal drug classes available as well as the unprecedented increase in antifungal drug resistance. A promising strategy to enhance antifungal efficacy and impede drug resistance is combination therapy, in which two bioactive compounds are combined. Protein kinases are key regulators of a variety of eukaryotic cellular processes and some studies suggest that targeting these signal transducers may be effective at combatting fungal infections. However, there has been limited analysis of the roles of fungal kinases in virulence and antifungal resistance, and the potential utility of kinase inhibitors in antifungal therapy remains largely unexplored. Employing a chemical biology approach, I screened 627 kinase inhibitors to look for molecules which inhibited C. albicans growth alone or in combination with the widely-used antifungal drug, fluconazole. In total, 10 and 18 compounds were identified with single agent or fluconazole potentiating activity, respectively. Hit compounds with low mammalian cytotoxicity profiles will be prioritized for mode-of-action studies using a combination of hypothesis-driven experiments and unbiased chemogenomic techniques. Overall, this project will characterize kinase inhibitors that possess antifungal activity in C. albicans and broaden the therapeutic target space for antifungal drug combinations.

53) Revision of Meropenem Zone Diameter Screening Breakpoint Reduces Unnecessary Confirmatory Testing for Carbapenemase Producing Enterobacteriacae

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Background: In light of the increasing need to detect carbapenemase producing Enterobacteriaceae (CPE) in clinical laboratories, EUCAST recently revised its carbapenem zone diameter breakpoints. Since October 2018, our laboratory discontinued screening by ertapenem disk diffusion and adjusted its meropenem zone diameter screening breakpoint to reflect this change. A retrospective analysis was conducted to estimate the effect these updated breakpoints would have had on CPE detection. Methods: From September 2015 to September 2018, our laboratory screened isolates for CPE based on the 2013 EUCAST zone diameter screening breakpoint of <25mm for meropenem or ertapenem (10µg disks). Isolates which screened positive were subjected to confirmatory phenotypic and molecular testing. For this study, we re-evaluated these previously screened positive isolates with the updated 2017 EUCAST meropenem screening breakpoint of <28mm, when accompanied by a temocillin 30µg disk zone diameter <11mm, and assessed the impact of discontinuing ertapenem screening on CPE detection. Results: 877 potential CPE isolates were detected from 417 (48%) screening and 460 (52%) clinical specimens using 2013 EUCAST breakpoints. 111/877 isolates were later confirmed CPEs, producing 766 false positive screens (positive predictive value, PPV 13%). If we defined CPE detection using only an ertapenem zone diameter <25mm, all 111 CPEs would be detected, but at a cost of 757 negative confirmatory tests (PPV 13%). Alternatively, if we defined detection using only a meropenem zone diameter <25mm, we would miss one OXA-48 producer while generating 159 negative confirmatory tests (PPV 41%). When using the 2017 EUCAST meropenem breakpoint coupled with temocillin testing, the missed OXA-48 was detected, and there was no difference in PPV (41%). Conclusion: Applying the 2017 EUCAST zone diameter screening breakpoints for meropenem and discontinuing ertapenem screening can reduce unnecessary CPE confirmatory testing by up to 80% without otherwise affecting the overall detection of CPEs identified in our laboratory.

54) Differential influences of complement on neutrophil responses to *Neisseria meningitidis* infection

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MD program from University of Würzburg visiting Gray-Owen lab, UofT

The complement system is the primary innate immune determinant protecting against invasive diseases caused by the Gram-negative bacterium Neisseria meningitidis (Nme, meningococcus), as evidenced by the extreme susceptibility of individuals with complement deficiencies. In contrast, the role of phagocytes such as neutrophils is much less well understood, although they are recruited in great numbers to the cerebrospinal fluid during meningococcal meningitis. Here, we consider the interaction of Nme with primary human neutrophils using either purified cells or a whole blood model of infection. We found that neutrophils are capable of non-opsonic uptake and killing of different Nme strains. However, in presence of immune serum featuring active complement, Nme-association is strongly increased, whereas this is not the case in heat-inactivated immune serum. Blockade of complement at the level of C3 using the inhibitor compstatin Cp20 reduces the uptake dramatically. In addition, purified neutrophils did not mount an oxidative burst towards Nme unless complement was added and, vice versa, the oxidative burst was strongly reduced in whole blood upon complement inhibition. In contrast, there was no significant impact of complement on neutrophil degranulation or IL-8 secretion. Taken together, neutrophils require complement activation in order to mount a full response towards Nme.

55) Each Additional Day of Antibiotics is Associated with Lower Gut Anaerobes in Neonatal Intensive Care Unit Patients

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Introduction

Infants in the neonatal intensive care unit (NICU) are often given extended doses of antibiotics. Antibiotics alter the normal acquisition of gut commensal and butyrogenic anaerobes. These changes in the normal development of the infant gut microbiota have been implicated in sepsis and necrotizing enterocolitis in the NICU as well as long-term health outcomes such as allergy, obesity, and inflammatory bowel disease. The drug and duration-associated effects of antibiotics need to be quantified to inform therapeutic decision-making in the NICU population.

Methods

Stool swabs were collected from NICU patients at The Hospital for Sick Children. Infants were included in the study if they received any exposure of ampicillin and tobramycin (AT), ampicillin and cefotaxime (AC), or ampicillin, tobramycin, and metronidazole (ATM). DNA was extracted from stool swabs and subject to Illumina sequencing of the V4 hypervariable region of the 16S rRNA gene. The first sample from each patient taken within one week after antibiotic exposure were analysed for taxonomic composition.

Results

A total of 72 infants were included in the study. Term infants received AT (20/28; 71%) or AC (8/28; 29%) with median durations of 3 and 3.5 days, respectively. Preterm infants received AT (32/44; 73%) or ATM (12/44; 27%) with median durations of 4 and 7 days, respectively. Compositional analyses of stool swab samples demonstrated low diversity and dominance by potential pathogens. Within a week of discontinuation of therapy, each additional day of antibiotics was associated with lower richness of obligate anaerobes (adjusted risk ratio = 0.83 [95% CI: 0.73 – 0.94]) and butyrate-producers (adjusted risk ratio = 0.78 [95% CI: 0.65 – 0.94]).

Conclusions

Each additional day of antibiotics is associated with lower richness of anaerobes and butyrate-producers within a week after therapy. A longitudinally sampled cohort with pre-exposure sampling is needed to validate our results.

56) The role of Nod2 signaling in mediating intestinal epithelial restitution

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Loss of function mutations in nucleotide-binding oligomizeration domain 2 (Nod2) is a strong genetic risk factor for the development of Crohn's Disease (CD). However, the role of Nod2 in CD etiology is still unclear. Nod2 is an intracellular innate immune receptor that detects muramyl dipeptide (MDP), a component of the bacterial cell wall. Nod2 signaling has been reported to play a role in intestinal stem cell survival and intestinal epithelial restitution. To investigate the role of Nod2 in mediating intestinal epithelial restitution, we used an irradiation-induced epithelial damage model. Our data demonstrate that Nod2 plays an active role in facilitating the recovery of the intestinal epithelium following damage. Intestinal epithelium-specific Nod2 knockout mice (Nod2ΔIEC) have decreased numbers of proliferating cells four days post-irradiation. To determine if Nod2 played a specific role in radioresistant intestinal stem cells, Bmi1-specific Nod2 knockout mice (Nod2ΔBmi1) were generated. While Nod2-deficiency in Bmi1-expressing cells did not impact the epithelial proliferative response to irradiation-induced injury, intestinal crypts isolated from Nod2ΔBmi1 mice had an impaired ability to form intestinal organoids. Single-cell RNA sequencing was performed to determine the impact of Nod2-dependent and independent signaling on the gene expression profile of differentiated cell types at baseline and three days post-irradiation. The preliminary analysis demonstrates the presence of intestinal epithelial cell types observed in published data sets and confirms that Nod2 expression is induced in recovery stem cells following irradiation. These data suggest that Nod2 signaling in intestinal stem cells play an important role in the recovery of the intestinal epithelium following damage.

57) Identification of novel therapeutic strategies to combat *Candida auris*

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The global burden of fungal disease has seen considerable growth in recent decades, with invasive fungal infections contributing to more than one million fatalities annually. Candida, a dominant pathogenic fungal genus, is a large contributor to the increased prevalence of human fungal infections, bolstered by the ever growing number of isolates of Candida species exhibiting resistance to the limited clinically available antifungal drug classes. In 2009, the emerging pathogenic Candida species Candida auris was first described and characterised. Since its discovery, C. auris has established itself as a global threat to public health, as infections are typically nosocomial with widespread resistance present across isolates to all three principal antifungal classes (azoles, echinocandins, and polyenes). Thus, there is a pressing need for development of novel treatment options that show efficacy against C. auris. The investigation presented here endeavours to identify novel strategies to target C. auris, in order to both broaden our understanding of its biology as well as to address the growing concerns that accompany its global spread. Leveraging a small molecule library-based chemical biology screening approach, two screens were completed to identify novel compounds with single agent bioactivity as well as potentiating activity of clinically available antifungals, fluconazole and caspofungin. These were performed with the Medicines for Malaria Venture's Pathogen Box compound library and Boston University's Centre for Molecular Discovery small molecule library. Following screening, eight compounds demonstrating previously unreported antifungal activity were validated through secondary assays, and prioritized for continued study. Further characterization of these bioactive molecules will involve screening each prioritized compound against a more diverse range of fungal pathogens, performing chemogenomic profiling, and conducting selection experiments to identify resistant mutations in order to determine the cellular target(s) of these compounds. Collectively, this work has the potential to unveil novel therapeutic strategies to combat fungal disease.

58) Identifying modulators of Slam-dependent SLP translocation

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Health practitioners around the world are fighting a losing battle against multi-drug resistant strains of bacteria that are the leading cause of hospital-acquired infections. In this study, we are investigating proteins that are anchored onto the cell surface of these bacteria via lipids called 'surface lipoproteins' (SLPs). These SLPs in Gram-negative bacteria have been a rising phenomenon and field of interest as they play essential roles in the pathogenesis of bacterial infections and have been shown to elicit bactericidal antibodies, making them prime candidates for the development of vaccines and antibiotics. The Moraes' lab discovered a novel set of integral outer membrane proteins named Surface Lipoprotein Assembly Modulator (Slam) that play a crucial role in translocating lipoproteins to the cell surface in Neisseria meningitidis1. The importance of Slam is illustrated in a knockout that renders the neisserial organism avirulent similar to the knockout of the virulence factor transferrin binding protein B (TbpB). These studies led to my research goal of identifying small molecules that inhibit Slam-dependent SLP translocation. To date, I have developed a cell-based assay and screened 149 crude extracts obtained from different strains of Streptomyces, which are known as principle source of antibiotics. From the screen, I have identified two top hit extracts that inhibited the surface display of TbpB. Future studies will include the purification of the active natural product and target deconvolution from these two hit crude extracts. This work is poised to reveal novel avenue for therapeutics that can specifically treat bacterial infections that cause sepsis, meningitis and chronic infections.

Reference:

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59) Host-Microbiota Interactions Shape the Intestinal Macrophage Landscape

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Intestinal macrophages (MΦs) are key immunological sentinels that locally maintain both tissue and immune homeostasis. This functional diversity and division of labor within the tissue are determined by their heterogenous developmental origins and requirements. Both, tissue- and microbiota-derived factors regulate intestinal MΦ differentiation, development, and distribution, yet these mechanisms remain poorly defined. Here, we identify two MΦ populations in the colonic lamina propria based on Tim-4 expression: fetal-derived Tim-4+ MΦs and adult monocyte-derived Tim-4- MΦs. Tim-4- MΦs, unlike Tim-4+ MΦs, preferentially reside within isolated lymphoid follicles and depend on Csf2 and Ccr2 for local maintenance. The abundance of Tim-4- MΦs decreases in germ-free and antibiotics-treated mice, indicating a requirement for microbial stimulation in monocyte-derived MΦ development. Increased size and diversity of the intestinal microbiota, as observed in neonatal mice or in adult mice colonized with commensal protozoan Tritrichomonas spp., drove monocyte infiltration and elevated numbers of Tim-4- MΦs in a CCR2-dependent manner. Collectively, our data demonstrate that microbiota diversity on the kingdom and species levels regulates the composition and abundance of MΦ subsets within the intestinal tract. Current research aims to address the molecular mechanisms regulating monocyte recruitment and the involvement of microanatomic niches in controlling MΦ heterogeneity.

60) Characterizing *Enterococcus faecalis* Induced Macrophage Dysfunction and Treatment with Bioactive Nanoparticles

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Background: Bacterial biofilms are composed of aggregates of microbial cells enclosed in a self-produced matrix adherent to a surface. Treatment of biofilm infections is currently a difficult and complicated challenge for microbiologists and clinicians. Antibiotic treatment alone is often inadequate to overcome biofilm infections and adjunctive therapies are required. Residual biofilm elucidates an inflammatory response at the host – biofilm interface. Macrophages are crucial modulators in the regulation of inflammation, and regeneration of numerous tissues. They play an important role in recognizing the molecular patterns on the bacterial cells, initiating the internalization, killing, and the production of cytokines. Macrophages could be polarized into pro-inflammatory and regulatory/anti-inflammatory phenotypes, depending on their exposure to different stimuli. Objective: Our aim is to understand the interaction of E. faecalis biofilm and macrophages and to evaluate the interaction of bioactive antibacterial nanoparticles treated-biofilm and macrophages. Hypothesis: Bioactive antibacterial chitosan nanoparticles (CSnp) would serve dual functions, inactivate residual biofilm and supress the proinflammatory response of macrophages. Methods: A clinically simulative ex-vivo model of mature E. faecalis biofilm in dentin was used to characterize the residual biofilm after conventional treatment with sodium hypochlorite or CSnp treatment using CFUs count, Confocal Laser Scanning Microscopy and Scanning Electron Microscopy. Secondly, the interaction of post-treatment residual biofilm in dentin and macrophages was evaluated in terms of pro/anti-inflammatory markers, and macrophages viability. Results: Microbiological analysis demonstrated that CSnp treated biofilm resulted in significantly less residual bacterial load with 3.7 log reduction compared to conventional treatment ($P<0.001$), and volumetric analysis of confocal images revealed 38.9% reduction in viable bacteria ($P<0.001$). Macrophages interacted with CSnp treated biofilm showed reduced pro-inflammatory markers (Nitric oxide, TNF-α and IL-6), increased anti-inflammatory marker (TGF-β1) and enhanced cells viability. Conclusion: CSnp have the potential to concurrently inactivate residual E. faecalis biofilm and suppress the pro-inflammatory response of macrophages.

61) FtsHP518S increase the translocation efficiency of Legionella pneumophila effectors by impacting the Dot/Icm T4BSS

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Legionella pneumophila is able to replicate within both amoebae and human alveolar macrophages, which is the causative agent of Legionnaires' disease. In order to maintain such a broad host-range, L. pneumophila evolved the largest effectors arsenal with over 350 effectors translocated by the Dot/Icm T4BSS. Limited access of intracellular iron to L. pneumophila is a critical arm of the nutritional immune response. To circumvent host iron restriction and gain access to iron across the host vacuolar, L. pneumophila employs Dot/Icm system to targets MavN to the surface of the Legionella-Containing Vacuole where it facilitates intra-vacuolar iron acquisition. MavN has an identified C-terminus translocation signal (TS) recognized by the Icm/Dot system, and □mavN displays severe intracellular growth defect which can be rescued only by adding excess exogenous iron to the culture medium. We have proposed a model in which effectors lack translocation signal are delivered to the host cell at a basal level by a relatively promiscuous Dot/Icm system. Such basal translocation would support the evolution of L. pneumophila to adapt to and replicate within hosts. At first, we measured the effects of removing translocation signals from MavN and observed an intermediate phenotype between wild-type and effector-null mutant. Then, we used experimental evolution to passage mavN(-TS) through host cells for several months to select for mutations that restore efficient translocation of MavN, or generate a more promiscuous Dot/Icm system. Adaptive mutations were identified through whole-genome sequencing. FtsH P518S were isolated and rescue the intracellular growth defect of mavN(-TS). Additionally, we verified that the translocation efficiency of MavN(-TS) and another effector lacking translocation signal LegC8(-TS) were increased in FtsH P518S. And P518S damage the protease activity of FtsH in some extent. Next, we'll aimed at determining the molecular mechanisms of FtsH P518S can overcome the MavN(-TS) phenotype.

62) Efficacy of bioactive nanoparticles on tissue-endotoxin induced suppression of stem cell viability, migration and differentiation

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Background: Regenerative endodontics aims at replacing inflamed/necrotic pulp tissue with dentin-pulp-like tissues to retain natural dentition and improve patient's quality of life. The ability of Stem Cells from Apical papilla (SCAP) to differentiate into odontoblast and secrete appropriate dentin matrix to strengthen the remaining root structure makes them an ideal candidate for regeneration procedures. Here, we characterize an LPS-treated dentin tissue model (LPS-dentin) to analyse the efficacy of polycationic chitosan nanoparticles (CSnp) and/or dexamethasone conjugate chitosan nanoparticles (Dex-CSnp) on the viability /differentiation potential of SCAP when exposed to LPS-dentin. We also aim at understanding, the effect of macrophage-dependent inflammation on SCAP migration in presence of LPS-dentin. Methodology: A total of 88 dentin slabs were used in the study. TOF-SIMS analysis was performed among the LPS-treated and untreated dentin groups (n=2/group). The study was conducted using four dentin groups: no treatment (control); LPS-treatment only; LPS-treatment followed by CSnp conditioning; and LPS-treatment followed by Dex-CSnp conditioning groups. SCAP adherence, viability, differentiation and biomineralization potential on dentin from different groups was studied using fluorescent and electron microscopy. Inflammation by macrophages in response to LPS-dentin was quantified and effect on SCAP migration was analysed. Statistical analysis was performed by using student's t-test with a significance level of $p<0.05$. Result: TOF-SIMS analysis confirmed LPS contamination. LPS-dentin affected SCAP viability but not adherence to dentin. Conditioning of LPS-dentin with either nanoparticles improved SCAP viability, and rescued other LPS related adverse effects on SCAPs, such as F-actin disruption, decrease in differentiation/biomineralization potential. IL-6 produced by macrophages in response to LPS- treated dentin impeded SCAP migration, diminished on CSnp and Dex-CSnp conditioning. Conclusion: This study developed an LPS-dentin model and highlighted the ability of CSnp and Dex-CSnp to promote stem cell viability, migration, differentiation potential and reduce inflammation, providing conducive environment for tissue regeneration/repair.

63) ILC2-controlled local eosinophilia along a protozoan-stimulated gut-lung axis

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Mucosal tissues such as the intestine and the lung are barrier sites exposed to the external environment. Barrier integrity and tolerogenic immunity are essential in sustaining homeostasis of these organs. Disruption in barrier function or immune homeostasis can increase susceptibility for inflammatory bowel disease and asthma. The mucosal immune system is interconnected, evident by the fact that immune responses mounted at a particular mucosal surface can provide protection at other mucosal sites. The intestinal microbiota is a key factor that shapes both the local and systemic mucosal immune system. Intestinal bacterial commensal composition is a known risk factor for asthma, however the effects of non-bacterial commensals remain to be explored. Protozoa are a common and integral part of the commensal microflora. While members of this kingdom can modulate intestinal immune responses, their role in modulating the systemic immune landscape remains poorly understood. Here, we identify an unexpected role for the protozoan commensal Trichomonas musculis (T.mu) in altering immune homeostasis across the gut-lung axis. Specifically, mice colonized with T.mu demonstrate increased eosinophilia within the bone marrow, blood, colon and lung. Intestinal colonization of mice with T.mu can activate intestinal group 2 innate lymphoid cells (ILC2) with minimal Th2 cell activation. Colonization of Rag2-deficienct (no T/B cells) with T.mu revealed a T cell-dependent systemic eosinophilia in the bone marrow and circulation, while colonization of Rag2 Il2rγ-double deficient mice (no-T/B/NK/ILC) uncovered a role for ILCs in supporting local eosinophil expansion. Interestingly, dietary supplementation with the T.mu-derived metabolite succinate can phenocopy colonization with T.mu, suggesting a succinate-dependent communication across the gut-lung axis as requirement for eosinophil homeostasis. Collectively, these results demonstrate that colonization with the commensal protist T.mu reshapes the immune microenvironment at distal mucosal sites. Current research investigates the effects of the protozoan-driven gut-lung axis in alveolar autoimmune pathologies and infections.

64) Trace levels of peptidoglycan in serum underlie the NOD-dependent cytokine response to endoplasmic reticulum stress

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NOD1 and NOD2 are intracellular sensors of bacterial peptidoglycan that belong to the Nod-like receptor (NLR) family of innate immune proteins. In addition to their role as direct bacterial sensors, it was proposed that NOD proteins could detect endoplasmic reticulum (ER) stress induced by thapsigargin, an inhibitor of the sarcoplasmic or endoplasmic reticulum calcium ATPase family (SERCA) that pumps Ca2+ into the ER, resulting in pro-inflammatory signalling. Here, we confirm that thapsigargin induces NOD-dependent pro-inflammatory signalling in epithelial cells. However, the effect was specific to thapsigargin, as tunicamycin and the subtilase cytotoxin SubAB from Shiga toxigenic Escherichia coli, which induce ER stress by other mechanisms, did not induce cytokine expression. The calcium ionophore A23187 also induced NOD-dependent signalling, and calcium chelators demonstrated a role for both intracellular and extracellular calcium in mediating thapsigargin-induced and NOD-dependent pro-inflammatory signalling, in part through the activation of plasma membrane-associated calcium release-activated channels (CRACs). Moreover, our results demonstrate that both endocytosis and the addition of serum to the cell culture medium were required for thapsigargin-mediated NOD activation. Finally, we analyzed cell culture grade fetal calf serum as well as serum from laboratory mice by high-pressure liquid chromatography and mass spectrometry, and identified the presence of various peptidoglycan fragments. We propose that cellular perturbations that affect intracellular Ca2+ can trigger internalization of peptidoglycan trace contaminants found in culture serum, thereby stimulating pro-inflammatory signalling. The presence of peptidoglycan in animal serum suggests that a homeostatic function of NOD signalling may have been previously overlooked.

65) Patterns-of-Practice Survey Demonstrates the Need for National Guidelines Regarding the Implementation of Microbiology Point-of-Care Tests Across Canada

Molly Lin(1), Antoine Corbeil(2), Robert Kozak(2), Elaine Kerr(3), Christie Vermeiren(2,5), Susan M. Poutanen(1,2,4), for the IQMH Microbiology Scientific Committee3

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Background: Microbiology point-of-care tests (POCT) are simple-to-use, automated assays performed outside of a laboratory infrastructure that can improve diagnostic accessibility and turnaround times, with potential benefits for antimicrobial stewardship and patient flow. Commercial availability and performance of POCT have rapidly evolved. Accreditation standards for implementing POCT exist for laboratories but if POCT are implemented without laboratory knowledge, these standards may not be followed. The goal of this survey was to describe laboratory awareness and involvement in POCT use.

Methods: In January 2018, a web-based patterns-of-practice qualitative survey was conducted by the Institute for Quality Management in Healthcare across all 73 laboratories participating in their bacteriology proficiency testing program. Questions addressed laboratory awareness and involvement in assuring accreditation standards were followed regarding POCT implementation.

Results: All 73 participants completed the survey. 12% of respondents reported POCT use within their hospital, while 5% reported adoption across affiliated outpatient settings. Notably, 11% and 18% were unsure, respectively. Of those aware of POCT use, 45% were not involved in the decision to introduce POCT on site, and 40% did not participate in the device selection process. Similarly, 40% of participants were unaware of any verification completed prior to the routine use of the device, and 20% noted the absence of standard operating procedures. 20% of participants noted absence of initial training and 30% were unaware of longitudinal competency assessments. Ongoing device maintenance was lacking in 89% of participating institutions. Additionally, 70% of participants stated that there was no overall monitoring of outcome measures after POCT implementation.

Conclusion: Our survey results indicate a low amount of laboratory awareness and involvement with microbiology POCT and a concerning proportions of institutions lacking standard quality management of POCT. In anticipation of the expanding adoption of POCT, establishing national guidelines requiring laboratory oversight of POCT should be a priority.

66) Severity of coronavirus virus respiratory infection in adults admitted to acute care

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Sunnybrook Health Sciences Centre

Recently, the WHO has highlighted the need for improved epidemiological surveillance and a better understanding of the health burden imposed by RNA respiratory viruses. Human coronaviruses (CoVs) are a major cause of respiratory and gastrointestinal infections with associated morbidity and mortality. The objective of our study was to characterize the epidemiology of CoV in our tertiary care health centre, and identify clinical correlates of disease severity. Methods: Nasopharyngeal and mid-turbinate swabs and bronchoalveolar lavages were tested for CoVs (OC43, 229E, NL63 and HKU1) by multiplex PCR (xTAG RVP, xTAG RVP FAST v2 or RPP, Luminex). Demographic and clinical data was obtained from the charts of patients admitted between 2010 and 2016, and statistical analysis s performed. A number of variables consistent with a severe disease burden were evaluated and included (but not limited to): patient outcome, ICU admission, number of symptoms and length of stay. Results: From 2010-2016 CoVs represented 11.2% (542/4660) of all positive respiratory virus samples. OC43 was the most commonly identified CoV, followed by 229E, NL63 and HKU1. The average length of stay for our cohort was 13.5 days, and it was noted that 17.5% required admission to the ICU (mean ICU admission time = 13 days). Overall mortality in our cohort was 7%, although no statistically significant difference in mortality or ICU admission was associated with any specific CoV strain. Conclusions: This study highlights the underappreciated burden of CoV in a hospital setting and suggests that more comprehensive study of CoV infections at the provincial and national level is necessary."

67) Verification of the NG-Test CARBA 5 Immunochromatographic Assay to Simultaneously Detect KPC, NDM, OXA48-like, VIM and IMP Enzymes in Species-Diverse Carbapenem-resistant Gram-Negative Bacilli

Bryn Hazlett, Yaroslav Sokolskyy, Pauline Lo, Tony Mazzulli, Allison McGeer, Susan M. Poutanen

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Objectives: Rapid detection of carbapenemase-producing organism (CPO) is essential for containment and patient management. We evaluated the ability of the NG-Test CARBA 5 immunochromatographic assay (NG Biotech, France) to detect five common carbapenemases using well-characterized Gram-negative bacilli (GNB): KPC, NDM, OXA-48/OXA-48-like, VIM, IMP.

Methods: 300 GNB including 260 CPO (250 targeted-CPO: 125 KPC, 71 NDM, 33 OXA, 10 NDM+OXA, 8 VIM, 3 IMP; 10 non-targeted CPO (4 GES, 5 SME, 1 NMC), and 40 non-CPO were tested comprising 292 Enterobacteriacaeae (115 Klebsiella pneumoniae, 79 Escherichia coli, 51 Enterobacter cloacae, 44 other) and 8 non-Enterobactericeae GNB (3 Pseudomonas species, 2 Acinetobacter baumannii, 2 Aeromonas hydrophila, and 1 Shewanella putida). Isolates were recovered from -80OC under selective pressure (MacConkey with ertapenem disc) and plated to Oxoid MacConkey-cefpodoxime/MacConkey-meropenem CPO screening bi-plates. As directed, a fresh 18h x 37OC colony of each isolate was collected with a loop and suspended in extraction buffer; 100µL of each was added to the sample well with results read at 15min x 21OC. The reader was blinded and discrepancies were repeated.

Results: Of 300 tests, results were easy to interpret and all but three (2 OXA, 1 mucoid KPC) were available within 5 minutes. CARBA 5 initially identified 245/250 targeted CPO-proteins 123/125 KPC (98.4%; 93.9-99.9); 79/81 NDM (97.5%; 90.1-99.8); 42/43 OXA (97.7; 86.8-99.9); 8/8 VIM (100%; 62.8-100); 3/3 IMP (100%; 38.2-100). Five targeted-CPO that were initially missed (2 KPC, 2 NDM, 1 OXA-48) were positive on repeat testing. The original missed KPCs were due to lost plasmids; the original missed NDMs were likely due to missed light bands; and the original missed OXA-48 was in the context of an NDM/OXA-48 positive isolate in which the NDM band showed up first. All 50 (91.5-100) non-targeted-CPO/non-CPO were negative. Final CPO-detection sensitivities/specificities were 100% for all targets: KPC 96.4-100/96.6-100; NDM 94.6-100/97.5-100; OXA 90.2-100/97.9-100; VIM 62.8-100/98.2-100; IMP 38.2-100/98.2-100.

Conclusions: The NG-Test CARBA 5 was easy to use and provided highly-accurate (100% sensitive/specific) rapid detection for KPC, NDM, OXA48-like, VIM and IMP CPO.

68) Verification of Three Multiplex Carbapenemase Nucleic Acid Amplification Tests (NAAT) using Species-Diverse Carbapenem-resistant Gram-Negative Bacilli (GNB)

Bryn Hazlett, Yaroslav Sokolskyy, Pauline Lo, Tony Mazzulli, Allison McGeer, Susan M. Poutanen

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Background: Rapid detection of carbapenemase-producing organisms (CPO) is important. Data suggest NAAT is more sensitive than culture. We evaluated three multiplex carbapenemase NAAT [BDMax Checkpoints CPO (BD), Allplex Entero-DR (Seegene), and Easyplex® SuperBug CRE Assay Version C (Amplex Diagnostics)].

Methods: 200 well-characterized (phenotypic and PCR/sequencing) GNB including 168 CPO (159 targeted-CPO: 94 KPC/30 NDM/8 OXA/15 NDM+OXA/9 VIM/3 IMP; 9 non-targeted CPO: 2 GES/4 SME/3 NMC), and 32 non-CPO GNB were tested. Discrepant results were repeated. Limit of detection (LOD) was calculated in triplicate using four QC strains following manufacturer direct-from-specimen-protocols using 10E4-10E8 cfu/L concentrations with results from Xpert® Carba-R (Cepheid) as reference. Colony counts confirmed concentrations and average LOD was calculated.

Results: LOD results are shown (Figure). The most sensitive to least sensitive assay was Seegene, BD, Cepheid, then Amplex. All 37 non-targeted-CPO/non-CPO were negative by all assays. 1 KPC and OX/1 OXA, NDM, and KPC/3 KPC were initially missed by BD/Seegene/Amplex, respectively but were positive on repeat testing of fresh subcultures suggesting initial lost plasmids. 2 IMP were reproducibly missed by BD and Amplex. Final CPO-detection sensitivities/specificities were 100% for all non-IMP targets; respective 95%CI were: KPC 94-100/95.3-100; NDM 96.5-100/89.1-100; OXA48-like 96.9-100/83.1-100; VIM 97.2-100/65.5-100. Sensitivities/specificities for IMP were 100%(38.2-100)/100%(96.2-100)(Seegene) and 33%(5.6-79.8)/100%(96.2-100)(BD&Amplex).

Conclusions: All three assays were highly-accurate for detection for KPC, NDM, OXA48-like and VIM CPO. IMP was more challenging for BD and Amplex. LOD was variable but not substantially different between assays. These results along with workflow, turn-around-time, footprint, interfaceability, cost, and laboratory needs can be used to determine suitability.

69) Impact of Picosalax and Multiple Fecal Microbiota Transplantations (FMT) by Enema on Microbiome Uptake in Patients with Recurrent *Clostridioides difficile* Infection (rCDI)

Jessica D. Forbes, Bassem Hamandi, Robbie Guang-Ye Jin, Melissa Kissoon, Susy Hota, Susan M. Poutanen

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Objectives: FMT is an effective treatment in patients with recurrent rCDI. Less is known regarding how the gut microbiota is influenced by bowel lavage prior to FMT and what impact multiple FMTs may have on the gut microbiota.

Methods: Nine rCDI patients undergoing FMT by enema were included in this study. Rectal ESwabs were obtained immediately before and after PICO-SALAX. Patients received 2-3 FMTs over a week following PICO-SALAX administration. Rectal ESwabs were obtained prior to the second and third as well as at 1-month following FMT. Rectal ESwabs were stored neat in -80°C. The MO BIO PowerSoil DNA Isolation Kit was used to isolate gDNA; 16s rRNA gene amplicon sequencing (V4 hypervariable region) was performed on the Illumina MiSeq. Low abundant OTUs (<0.001%) were excluded from the dataset. Microbiota measures were compared using Wilcoxon signed-rank test with Benjamini-Hochberg false discovery rate correction.

Results: There were no significant differences in proportion of phyla or genera in the pre- versus post-PICO-SALAX microbiota but there were significant decreases in richness (ACE, Chao1) and number of observed taxa and increases in the diversity (Simpson and Shannon). Significant differences in the patient's baseline microbiota (increases in Bacteroidetes and Proteobacteria but decreases in Actinobacteria and Fusobacteria) were observed after three FMT at the 1-month follow-up but not after the first nor second FMT. While changes in alpha diversity were observed post-FMT, none were considered significant.

Conclusions: The microbiota was only significantly changed consistently at the 1-month follow-up suggesting the need for multiple FMT when delivering FMT by enema. Bowel lavage with PICO-SALAX significantly reduced the number of observed taxa and modified diversity indices but did not otherwise have a substantive impact on the microbiota. Whether bowel lavage is needed to improve uptake of donor microbiota requires further evaluation.

70) Impact of Storage Conditions of Stool and Fecal Filtrate on Microbiome Composition – Implications for Microbiota Researchers and Fecal Microbiota Transplantations

Jessica D. Forbes, Bassem Hamandi, Robbie Guang-Ye Jin, Melissa Kissoon, Susy Hota, Susan M. Poutanen

University of Toronto - Department of Laboratory Medicine and Pathobiology, University of Toronto - Leslie Dan Faculty of Pharmacy, University Health Network, Sinai Health System, University of Toronto - Department of Medicine

Objectives: Stool storage is key to researching the association between gastrointestinal dysbiosis and disease states. Fecal microbiota transplantation using frozen filtrate is used for patients with recurrent Clostridioides difficile infection. This study determined the impact of storing stool and frozen filtrate on microbiome composition.

Methods: Fresh stool was obtained from a high-diversity (HD) and low-diversity (LD) donor. Aliquots were stored at room temperature (RT), 5°C, and at -20°C for 24 and 48 hours, or processed immediately. Fresh stool was also homogenized with both 0.9N-sterile-saline and 0.9N-sterile-saline containing 10%-glycerol. Resulting filtrate aliquots were frozen at -20°C and at -80°C. At baseline and after 7, 9, 12, 18 and 24M storage, gDNA was isolated using the MO BIO PowerSoil® DNA Isolation Kit. 16s rRNA gene amplicon sequencing targeting the V4 hypervariable region was performed on the Illumina MiSeq. Low abundant OTUs (<0.001%) were excluded from the dataset.

Results: Differences in microbiota profiles were observed in both HD and LD stool when stored immediately at –20°C, more so with the LD stool. Differences were also noted for LD stool stored at room temperature and less so at 5°C. Storage at RT or 5°C had no impact for the HD stool. Long-term filtrate storage of the HD stool filtrate at –80°C with 10%-glycerol best preserved the bacterial microbiota profile followed by –20°C with 10%-glycerol then –80°C and –20°C without 10%-glycerol. Differences were also observed in the LD stool filtrate when stored in any conditions, with the least impact being –80°C with glycerol.

Conclusions: To preserve microbiota profiles, storing stool at 5°C until received in the laboratory is optimal. For researchers assessing microbiota correlations with disease states, immediate gDNA extraction from stool upon receipt is ideal. For those using frozen filtrate from healthy donors for fecal transplants, long-term storage is best at –80°C with 10%-glycerol.

71) Geo-temporal Epidemiology of Extended-spectrum Beta-lactamase (ESBL)- producing ST131 Escherichia coli isolated from Bloodstream Infections in the Toronto Region

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University of Toronto, Public Health Ontario, University Health Network/Sinai Health System, University Health Network, St. Michael's Hospital

Objectives: ESBL-producing Enterobacteriaceae are an increasing threat. We have described the rise in ESBL-producing E. coli in the Toronto region. Herein, we describe the associated multi-locus sequence types (MLST) and geo-temporal epidemiology using whole genomic sequencing (WGS) of bloodstream ESBL-producing E. coli.

Methods: All adult inpatients from four tertiary-care hospitals in Toronto with bloodstream ESBL-producing E. coli from 2006 through 2012 were included (n=174). Corresponding isolates, one/patient/year, were recovered from -80C and genomic DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen). WGS was completed on the NextSeq500 with 2x150 base paired-end reads using the Nextera XT DNA Library Prep Kit (Illumina). Genomes were denovo assembled with SPAdes. MLST were determined using the Bacterial Analysis Pipeline with MLST 2.0.1 (Center for Genetic Epidemiology). Linear trend analysis and geotemporal mapping using the patient's residential postal code was completed using Tableau.

Results: There was a significant rise in the total number of bloodstream ESBL-producing E. coli between 2006 and 2012 (P=0.0005) with a corresponding rise in the proportion of ESBL-producing E. coli from 6.4% to 12.7%. This rise was driven by a significant rise in ST131 (P=0.0002) (Figure), with significant rises noted in three hospitals (P=0.004, 0.02,0.01) and a non-significant trend in the fourth (P=0.08). The change in non-ST131 E. coli was not significant (P=0.09) (Figure). ST131 E. coli was first noted in patients residing in Brampton, a city in which 30.6% identify as having East Indian ethnicity (Figure), notable as the proportion of ESBL-producing E. coli has been reported as high as 70% in South East Asia.

Conclusions: There was a significant rise in bloodstream ESBL-producing E. coli in the Toronto region between 2006 and 2012 led by the introduction and increase in ST131 E. coli. Further characterization of the factors associated with the endemicity of ST131 is ongoing.

72) Molecular epidemiology of nosocomial HCoV-OC43 strains responsible for outbreaks in a veteran population

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Background: Human coronaviruses (HCoVs) represent a common cause of common respiratory illnesses and outbreaks. As of yet, no studies have been conducted in the Canadian context towards determining the variability of circulating strains.

Objectives: To characterize the molecular epidemiology of the HCoV-OC43 strains responsible for the long-term care home (LTCH) outbreaks based on variability in the spike (S) gene.

Methods: Mid turbinate swabs (MTS) were collected from LTCH patients within 24 hours of showing symptoms of respiratory infections. Two non-roommates who tested positive for HCoV-OC43 were defined as outbreak patients and included in study criteria. Samples were then sequenced for the complete S gene to determine variants among circulating strains. Bayesian phylogenetic analysis and normalized polymorphism scores across the S gene were used to characterize variability.

Results: Outbreaks from 2015 and 2016 were likely caused by one introduction into the veteran population. Comparatively, the two genetically distinct variants from 2017 indicate that there were at least two HCoV-OC43 introductions into the LTCH that resulted in outbreaks. Phylogenetic analysis revealed that 2015 outbreak strains were genetically distinct from 2016 and 2017 samples, suggesting local circulation of distinct clades from 2015-2017.

Conclusions: The S gene of strains circulating within outbreaks were generally well-conserved, and indicate outbreaks were caused by one introduction into the veteran community which then transmitted from host-to-host. The variation between outbreaks suggest there are more viral mutational events occurring within the larger community, giving rise to multiple subtypes.

73) Duration of colonization with Carbapenemase-producing Enterobacteriaceae (CPE): a population-based study

Lubna Farooqi, Alainna Jamal, Amna Faheem, Zoe Zhong, Irene Armstrong Emily Borgundvaag, Brenda Coleman, Karen Green, Kithsiri JayasingheJennie Johnstone, Kevin Katz, Philipp Kohler, Angel Li, Roberto Melano,Matthew Muller, Sarah Nayani, Samir Patel, Aimee Paterson, Susan Poutanen,Anu Rebbapragada, David Richardson, Alicia Sarabia, Shumona Shafinaz,Andrew Simor, Barbara Willey, Laura Wisely, Allison McGeer

INFECTIOUS DISEASE RESEARCH UNIT-MOUNT SINAI HOSPITAL

Objectives: We aimed to determine the duration of CPE colonization to better inform providers, patients and infection control programs about prognosis.

Methods: Participants were recruited from population-based surveillance. Eligible persons were colonized/infected with CPE, had stable housing, and were expected to survive and reside in the area for ~1year. Participants were screened (groin & rectal swabs, and urine samples) at 1 and 3 months after identification, then quarterly until 3 negative sets of screening specimens were obtained. Specimens were incubated in BHI broth overnight then subjected to direct PCR to identify carbapenemase genes, with culture of PCR positive specimens. Decolonization was defined as occurring when 3 complete sets of swabs were PCR negative, and no later swabs were positive or clinical isolates identified. Time of decolonization was defined as date of first qualifying negative swabs.

Results: 284 (76%) of 385 eligible persons participated: 87 completed follow-up, 113 are being followed, and 84 have incomplete data (30 died; 54 withdrew/were lost to follow-up). Median age is 70 years, 164 (58%) are male, 182 (64%) have at least one underlying comorbidity, and 99 (35%) initially had a clinical specimen (vs. screen only). Most common organisms were E. coli (152,54%), Klebsiella spp. (92,32%) and Enterobacter spp. (25,9%); most common genes were blaNDM (±OXA) (164,58%), blaKPC (24,8%), and blaOXA-48-like (82,29%). The figure shows time to decolonization. Men (OR 0.53 95%CI 0.34,0.81), persons colonized/infected with Klebsiella spp. versus other bacteria (OR 0.50, 95%CI 0.24,1.02), those with clinical isolates (OR 0.49, 95%CI 0.29,0.81), and those with more sites positive at enrolment (OR for 2v1 site 0.30, 95%CI 0.14,0.67) were less likely to become decolonized.

Conclusions: Most CPE colonized/infected persons appear to clear their organism over time, although about 1 in 5 remain colonized at 2 years. Individual characteristics significantly affect duration of colonization.

74) Duration of colonization with Carbapenemase-producing Enterobacteriaceae (CPE): a population-based study

Lubna Farooqi, Alainna Jamal, Amna Faheem, Zoe Zhong, Irene Armstrong Emily Borgundvaag, Brenda Coleman, Karen Green, Kithsiri JayasingheJennie Johnstone, Kevin Katz, Philipp Kohler, Angel Li, Roberto Melano,Matthew Muller, Sarah Nayani, Samir Patel, Aimee Paterson, Susan Poutanen,Anu Rebbapragada, David Richardson, Alicia Sarabia, Shumona Shafinaz,Andrew Simor, Barbara Willey, Laura Wisely, Allison McGeer

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Conclusions: Most CPE colonized/infected persons appear to clear their organism over time, although about 1 in 5 remain colonized at 2 years. Individual characteristics significantly affect duration of colonization.

75) A Systematic Review of Solid Organ Transplantation in Acute Presentations of Tropical Infectious Diseases

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Fulminant life-threatening presentations of acute tropical infections such as yellow fever, dengue, malaria, hepatitis E, and leptospirosis, may occur, and the degree of end-organ impairment may qualify patients for solid-organ transplantation (SOT) in centres with such capacity. However, due to a paucity of synthesized data, there is a knowledge gap around indications for and outcomes in SOT for severe acute tropical infectious diseases. We therefore aim to synthesize such knowledge, focusing on patient outcomes in order to inform triage and treatment protocols in centres where acute tropical infectious diseases and SOT capacity may intersect. Five electronic databases were searched (PubMed, Embase, Scopus, Cochrane, and LILACS) using combinations of search terms such as the following: “liver” or “hepatic” “transplant,” “yellow fever” “dengue” and “Plasmodium spp.,” from database inception to March 4, 2019. A total of 6317 articles were retrieved: 2324 articles on PubMed, 3839 on Embase, 244 on Scopus, 43 on Cochrane, and 108 on LILACS. After eliminating duplicates using Mendeley software, a total of 4944 articles remained for title screening. Titles, abstracts, and full-text articles will be systematically double screened by two reviewers with a tertiary arbitrator. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) will be implemented. Data extraction will be performed by two reviewers and the quality of the articles will be critically evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. The data will be summarized to systematically map published literature that will illuminate the frequency, indications for, and health outcomes of SOT recipients in the treatment of acute tropical infectious diseases. Where SOT capacity exists alongside the occurrence of endemic or imported tropical infectious diseases, such synthesized information, particularly in the form of a clinical resource, is essential for appropriate resource allocation and informed clinical decision-making.

76) Development of Fungal-Selective Molecules and Strategies to Target Hsp90

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The frequency of invasive fungal infections is on the rise, and new therapeutic strategies are imperative as the three major classes of antifungals are hampered by host toxicity, the emergence of drug resistance, and a narrow activity spectrum. Selective targeting of stress responses in fungal pathogens provides a promising therapeutic strategy to mitigate drug resistance and combat invasive mycoses. The molecular chaperone Hsp90 has been extensively validated as an essential regulator of virulence traits and antifungal resistance in Candida species, but toxicity of the current Hsp90 inhibitors, that inhibit the host chaperone, impedes their use as antifungal treatments. Here, we assess the therapeutic potential of targeting Hsp90 and its regulatory circuit in fungal pathogens. To do so, we are performing structure-guided optimization of diverse analogs of a natural product Hsp90 inhibitor, radicicol. Ligand binding is assessed by a fluorescence polarization-based assay in fungal and human lysates, and molecules with fungal-selectivity are investigated for bioactivity in whole cell assays against C. albicans and another leading fungal pathogen, Cryptococcus neoformans. Through the screening of >160 synthetic compounds, we have identified one with 21-fold fungal binding selectivity and greater potency than radicicol against C. neoformans. In parallel, we aim to identify Hsp90-interacting proteins that govern stress responses and virulence that are fungal-specific. Extending our previous studies of chemical genetic Hsp90 interactors in C. albicans, we have screened ~4,000 deletion mutants to map the Hsp90 chemical genetic interaction network in C. neoformans, revealing nineteen robust interactors, including components of the ergosterol biosynthesis and COP9 signalosome pathways. Together, this work identifies novel components of core circuitry governing cellular stress responses and strategies to cripple fungal pathogens.

77) Rifampin-Ofloxacin-Minocycline (ROM) for the Treatment of Paucibacillary Leprosy: A Systematic Review

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Leprosy is a complex tropical infection from a diagnostic and management perspective, as patients with leprosy are at risk of numerous related complications from the disease itself and its treatment. Standard WHO multi-drug treatment (MDT) consists of medications that are potentially harmful and cause a range of adverse systemic effects. Monthly- or single dosing of ROM has emerged as a potential treatment option for leprosy, however, a synthesis of the evidence supporting ROM does not exist. Paucibacillary leprosy, characterized by limited skin lesions and a low bacillary load, may be most amenable to a fluoroquinolone-based treatment protocol. We performed a systematic review of relevant literature to evaluate the safety and efficacy of ROM-based treatment for paucibacillary leprosy. Various databases were searched from inception to March 2019, using a combination of search terms “leprosy”, “rifampin”, “ofloxacin”, “minocycline”, and “ROM”, while also accounting for alternative disease and chemical identifiers. The systematic review will focus on assessing and reporting on the efficacy, and safety of monthly ROM in the treatment of paucibacillary leprosy within a human population. 1139 records were retrieved for title and abstract screening, however, after a multi-step de-duplication pipeline, 568 articles remained. Subsequent title screening yielded 288 studies that were eligible for final inclusion. Main outcome measures to be assessed are lesion clearance, treatment failure, relapse, side effects and reversal reactions. A cursory review of relevant abstracts suggests that important determinants of health in the treatment of leprosy are: social environments, patient education, health services, gender and income. Synthesizing the current evidence discussing the efficacy of monthly ROM, will strengthen the current body of knowledge surrounding the treatment of paucibacillary leprosy, and may allow for the development of standardized fluoroquinolone-based treatment protocols.

78) Ethnopharmaceuticals for the Treatment of New World Cutaneous Leishmaniasis: A Systematic Review of Topical Application of Pepper and Allium

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New World Cutaneous Leishmaniasis (NWCL) is a neglected parasitic disease caused by members of the genus Leishmania primarily identified in Central and South America. Better drugs are urgently needed given the toxicity, expense and accessibility limits of first-line treatment options. Plant-based compounds with potential anti-leishmanial effects found in and around local endemic communities, particularly in and around the Amazon basin, present an opportunity to overcome the aforementioned therapeutic challenges, and many such interventions are supported by anecdotal evidence of efficacy. We aim to synthesize existing evidence around available ethnopharmaceuticals to promote drug discovery for the prevention and treatment of NWCL. PubMed (NCBI), Medline (OVID), Embase (OVID), Web of Science (BioSIS) and LILACS (VHL) were searched for from inception to July 26, 2018 using combinations of the search terms "cutaneous leishmaniasis" and "ethnopharmaceuticals". Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. For the systematic review, we included molecular, mechanistic, and observational studies, case reports, case series, cohort studies, as well as clinical trials reporting therapeutic outcomes, if possible using the GRADE approach. A total of 13667 abstracts were retrieved, after which 7566 duplicates were removed. Of the remaining abstracts, 550 abstracts were included in the full text review, of which 176 (32%) abstracts highlighted New World species; 116 (66.0%), 33 (18.7%), and 27 (15.3%) abstracts pertained to L. amazonensis, members of Viannia subgenus, and other New World species, respectively. Of all the abstracts included in the full text review, 25 (4.5%) and 6 (1.1%) were identified for Piper spp. "Pepper" and Allium spp. "Garlic", respectively. Synthesizing the current evidence surrounding ethnopharmaceuticals for the treatment of NWCL may contribute to drug discovery pipelines and potentially lead to novel therapeutics, particularly those targeting the Viannia complex, where patients often develop more severe clinical manifestations.

79) Lessons from Neisseria: HIV Shock-and-kill using Heptose phosphates

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With the advent of antiretroviral therapeutic agents and their combination (Highly active antiretroviral therapy, HAART), HIV has become a manageable infection1. However, accumulating toxicities and inability to achieve a cure by HAART has fueled research to achieve drug free remission of HIV. 'Shock-and-kill' proposes the use of latency reversing agents in the context of HAART to awaken latent HIV from reservoirs and reveal them to immune responses2. Clinical trials with candidate latency reversing agents did not reduce viral reservoirs3. Furthermore, LRAs had a negative impact on relevant immune responses and off-target toxicities, reducing the enthusiasm for their use4. Thus, improved latency reversing compounds must be developed. Clinical and epidemiological studies indicate that a synergy exists between Neisseria gonorrhoeae (Ngo), and HIV5, which increases susceptibility to and infectiousness of HIV6 might provide novel avenues of therapy. Our group discovered that heptose-1,7-bisphosphate (HBP) synthesized and secreted by Ngo can revert HIV out of latency8. Further studies have elucidated the potential role for Heptose-phosphates in engaging immune responses9. We hypothesize that HBP could be used as an LRA through its latency reversal and immune stimulating activities. We have shown through in vitro models of HIV latency that HBP is superior than recognized LRAs in reverting HIV latency. Additionally, HBP was safer to administer compared to conventional LRAs in vitro. Preliminary work shows that HBP can synergize with LRAs, suggesting alternative formulation strategies. Thus, HBP shows promise as a safe and effective LRA for shock-and-kill therapy.

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80) Intercurrent Flaviviral Viremia in Ill Returned Travelers with *Plasmodium vivax* Malaria

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Background: Similar epidemiology and clinical presentations of arboviral infections and malaria coupled with the typically sequential approach to diagnostic testing, where malaria is confirmed or excluded urgently in febrile returned travelers, may mask the true epidemiology of co-infections. Flaviviruses are known to trigger relapsing forms of malaria, including Plasmodium vivax, long after primary malaria infection, and this may delay the diagnosis of malaria.

Objective: We aim to understand the incidence of intercurrent flaviviral infection in confirmed Plasmodium vivax infection.

Method: DNA and RNA from biobanked isolates of P. vivax detected in whole blood at the Public Health Ontario Laboratory between 2006 and 2019 were extracted and screened for intercurrent flaviviral infections using previously validated real-time PCR (qPCR) assays targeting multiple flaviviruses (pan-FLAV) and, specifically, dengue virus types 1-4 (DEN1, DEN2, DEN3, DEN4).

Results: Five hundred and two unique isolates of P. vivax were identified, of which 175 have been tested to date. Males accounted for 69.1% (n=121/175) of P. vivax cases, while females accounted for 28.6% (n=50/175), and sex was unassigned in 2.3% (4/175). Median age of P. vivax cases was 34.2 years (range 3.7 years – 87.6 years; IQR 24.0 – 51.9 years). Median parasitemia was 0.1% (range < 0.01% - 1.1%). Sixty-eight (38.9%) P. vivax cases had documented travel history exclusively to South Asia, with India as the most common source country (34/175 [19.4%]). Pan-FLAV assay yielded a 0.6% (1/175) positivity rate. DENV assay yielded a 0.6% (1/175) positivity rate. Type-specific real-time PCR revealed DEN1, which was also detected on both Pan-FLAV and pan-DENV assays.

Conclusion: Intercurrent flaviviral viremia, was noted in at least 0.6%, which may suggest that primary flaviviral infection, in this case, DEN1, triggered a relapse of P. vivax. Alternatively, such co-occurrence may suggest primary infection with both organisms known to cause fever in returning travelers. Consideration of flaviviral coinfection should be given to the P. vivax patient with deep thrombocytopenia, lymphopenia, and high-yield arboviral symptomatology such as rash and retro-orbital headache.

81) Intercurrent Flaviviral Viremia and Plasmodium ovale Infection in Ill Returned Travelers to Ontario

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Similar epidemiology and clinical presentations of arboviral infections and malaria coupled with the typically sequential approach to diagnostic testing, where malaria is confirmed or excluded urgently in febrile returned travelers, may mask the true epidemiology of co-infections. Flaviviruses are known to trigger relapsing forms of malaria, including Plasmodium ovale, long after primary malaria infection, and this may delay the diagnosis of malaria. We aim to understand the incidence of intercurrent flaviviral infection in confirmed Plasmodium ovale infection.DNA and RNA from biobanked isolates of P. ovale detected in whole blood at the Public Health Ontario Laboratory between 2006 and 2019 were extracted and screened for intercurrent flaviviral infections using previously validated real-time PCR (qPCR) assays targeting multiple flaviviruses (pan-FLAV) and, specifically, dengue virus types 1-4 (DEN1, DEN2, DEN3, DEN4). One-hundred seventeen unique isolates of P. ovale were identified, of which 68 had sufficient remaining specimen for further molecular analysis. Males accounted for 54.4% (n=37/68) of P. ovale cases, while females accounted for 44.1% (n=30/68), and sex was unassigned in 4.4% (3/68). Median age of P. ovale cases was 27.4 years (range 22 mos - 72 years; IQR 18.8 – 40.1 years). Median parasitemia was < 0.01% (range < 0.01% - 0.8%). Thirty-one (45.6%) P. ovale cases had documented travel history exclusively to Africa, with Nigeria as the most common source country (23/31 [74.1%]). Pan-FLAV assay yielded a 1.6% (1/68) positivity rate. DENV was not detected in any specimen. P. ovale infections are most commonly imported to Ontario from West Africa, and Nigeria, specifically. Intercurrent flaviviral viremia was noted in at least 1.6%, which may suggest that primary flaviviral infection triggered a relapse of P. ovale. Alternatively, such co-occurrence may suggest primary infection with both organisms known to cause fever in returning travelers. Consideration of flaviviral co-infection should be given to the P. ovale patient with deep thrombocytopenia, lymphopenia, and high-yield arboviral symptomatology such as rash and retro-orbital headache.

82) Novel Detection of Leishmania RNA Virus-1 (LRV-1) in Leishmania Viannia panamensis Clinical Isolates

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American tegumentary leishmaniasis (ATL) comprises a discrete set of clinical presentations of leishmaniasis endemic to Central and South America. Leishmania RNA virus-1 (LRV-1) is a double stranded RNA virus identified in 20-25% of the Leishmania (L.) Viannia (V.) braziliensis and L. V. guyanensis, and is believed to be a predictive biomarker of severe ATL. To date, LRV-1 has loosely been described in other members of the Viannia complex including L. V. peruviana and L. V. lainsoni, however not in L. V. panamensis. We describe the novel detection of LRV-1 in L. V. panamensis and its associations with clinical phenotypes of ATL. Unique surplus discard clinical isolates were identified from Public Health Ontario Laboratory (PHOL) and the Leishmania Clinic of the Instituto de Medicina Tropical “Alexander von Humboldt” between 2012 and 2018. Banked clinical isolates were species identified by PCR, RFLP analysis, and Sanger sequencing. Clinical isolates identified as L. V. panamensis were screened for LRV-1 by real-time PCR. Patient isolates were stratified according to clinical phenotype: localized cutaneous leishmaniasis (LCL) was defined as "non-severe" ATL, whereas "severe ATL" was defined as mucosal or mucocutaneous leishmaniasis (ML/MCL); erythematous, purulent, or painful ulcers and/or lymphatic involvement (inflammatory ulcers); or multifocal/disseminated ulcers (≥ 4 in ≥ 2 anatomic sites). Of 22 patients with confirmed L. V. panamensis, 9 (41%), 7 (32%), 5 (23%), and 1(0.5%) had travel history to or resided in: Peru, Costa Rica, Ecuador and Panama, respectively. Nine (41%) and 13 (59%) patients had the severe and non-severe phenotypes, respectively. Three (33%) of 9 severe cases and 4 (30.8%) of 13 non-severe cases were positive for LRV-1, respectively ($p=0.90$). Median age of patients did not differ by clinical phenotype (median age 45.75 years in severe ATL vs. 31.93 years in non-severe ATL, $p=0.09$), or LRV-1 status (median age 34.14 years in LRV-1 positive patients vs. 38.27 years in LRV-1 negative patients, $p=0.64$). No differences in sex were observed for clinical phenotype ($p=0.60$) and LRV-1 status ($p=0.52$). Although an association between LRV-1 status and clinical phenotype was not demonstrated, we describe the novel detection of LRV-1 in L. V. panamensis, a species that has been documented predominantly in Central America. The role of LRV-1 in severe disease of L. V. panamensis requires further exploration to understand the dynamics of influencing host-immune responses as observed by L. V. braziliensis and L. V. guyanensis.

83) Ethnopharmaceuticals for the Treatment of Old World Cutaneous Leishmaniasis: A Systematic Review of Topical Application of Tumeric

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Toxicity, expense, and accessibility limit treatment success in Old World Cutaneous Leishmaniasis (OWCL), a neglected parasitic disease caused by members of the genus Leishmania found in the Middle East, Mediterranean basin, Arabian Peninsula, Africa as well as the Indian Subcontinent. Better drugs are urgently needed, however, drug discovery is hindered by limited funding given geographic restriction of highly endemic OWCL to LMICs. Plant-based compounds with potential anti-leishmanial effects found in and around local endemic communities present an opportunity to overcome the aforementioned therapeutic challenges, and many such interventions are supported by anecdotal evidence of efficacy. We aim to synthesize existing evidence around available ethnopharmaceuticals to promote drug discovery for the prevention and treatment of OWCL. PubMed (NCBI), Medline (OVID), Embase (OVID), Web of Science (BioSIS) and LILACS (VHL) were searched for from inception to July 26, 2018 using combinations of the search terms "cutaneous leishmaniasis" and "ethnopharmaceuticals". Iterative inclusion and exclusion of search terms was employed to maximize relevant article extraction. The GRADE approach will be used to assess quality of studies reporting therapeutic interventions. 3057 PubMed, 2818 Medline, 4200 Embase, 3183 Web of Science and 490 LILACS articles were retrieved for title and abstract screening; after duplicate removal, 5492 remained. 550 abstracts met inclusion criteria for full-text review, of which, 241 (43.80%) abstracts pertained to Old World species, and 113 (21%) were specific to L. donovani. Curcuma spp. “Turmeric” was identified in 4 articles (0.7%) to date. Synthesizing the current evidence surrounding ethnopharmaceuticals for the treatment of OWCL may contribute to drug discovery pipelines and potentially lead to novel therapeutics in a field that has not seen any new drug development for over half a century, especially in the context of turmeric.

84) Management of Common Intestinal Parasites in Pregnancy: A Systematic Review of Fetal and Neonatal Outcomes

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Parasitic infections in pregnancy necessitate consideration of numerous factors including potential maternal-to-child parasite transmission risk during pregnancy and delivery and potential anti-parasitic drug toxicity to the fetus and development of the newborn. We aim to map the available literature regarding the efficacy, safety, and tolerability of treatment of intestinal parasites in pregnancy, and synthesize the available literature on specific parasitic infections and anti-parasitic agents. 2479 articles were identified and 1774 articles were retrieved for title, abstract and full-text screening. Two independent reviewers with a tertiary arbitrator screened all systematic reviews, randomized controlled trials, cohort studies, smaller observational studies, case-control studies, case series, and case reports assessing or reporting the efficacy, safety, or tolerability of anti-parasitic drugs used in management of parasitic infections during pregnancy. Two independent reviewers extracted the data and assessed trial quality using the GRADE approach. Data were summarized using qualitative and quantitative measures for specific parasitic infections as well as efficacy and safety of anti-parasitic agents. Risk of bias for each study was determined. With increased international travel and migration of migrant and vulnerable populations, it can be expected that health practitioners will be faced with managing parasitic infections in pregnant patients. Currently, quality evidence supporting specific management strategies is limited. Synthesizing the current literature on anti-parasitic agents and treating parasitic infections in pregnancy can translate into multidisciplinary clinical recommendations for improved pregnancy care.

85) Sequence Heterogeneity in Leishmania RNA Virus-1 (LRV-1) Detected in Strains of *Leishmania Viannia* spp.

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Alexander von Humboldt", Lima, Peru 4Tropical Disease Unit, UHN-Toronto General Hospital, Toronto, ON, Canada Leishmania RNA Virus (LRV) is classified as a Group III dsRNA virus belonging to the family Totiviridae, containing a 5284 nucleotide sequence. Two main types of LRV are known to infect strains of Leishmania: LRV-1 and LRV-2. LRV-1 in the New World has 14 subtypes (LRV-1-1 – LRV-1-14) predominantly isolated from the Amazon basin. Since the detection of LRV-1 in a patient with cutaneous satellite lesions and lymphatic involvement after visiting Suriname, the notion that LRV-1 in the parasite might be causing more severe disease has been the focus of evaluation over the past few decades. We wanted to understand whether sequence heterogeneity within the LRV-1 virus could contribute to the severe phenotype observed in some patients infected by the Leishmania Viannia spp. Nucleic acid was extracted from clinical cultured cells for species identification and LRV-1 detection using quantitative real-time PCR (qPCR). Cultures positive by qPCR were confirmed by end-point PCR and Sanger Sequencing using primers targeting LRV-1-1 and LRV-1-4 subtypes primarily known to circulate in Latin America. Of 56 available clinical cultures, 18 were positive by qPCR. To date, 3/18 (16.7%) LRV-1 positive clinical cultures have been confirmed by end-point PCR with sufficient sequence product. The following species were identified: 2/3 (67%) L. V. braziliensis and 1/3 (33%) L. V. panamensis. A phylogenetic molecular analysis was performed using the Maximum Likelihood method (BioEdit version 7.2.5) post ClustalW Multiple alignment using the three previously mentioned clinical cultures, ATCC© 50126 L. V. guyanensis and NCBI reference genomes: NC002063.1 and NC00306.1. An unrooted tree was produced 2 distinct clusters whereby the 2 LRV-1 positive L. V. braziliensis and 1 ATCC© 50126 L. V. guyanensis strains were branched from the same node (length = 0.08639, $p < 0.01$) while the LRV-1 positive L. V. panamensis, NC00306.4 and NC002063.1 strains branched from an unrelated node (length = 1.23789, $p < 0.01$). Further analysis of the remaining LRV-1 positive cultures will provide more insight into the divergence of LRV-1 between species as well as implications into the severity of disease in ATL.