2nd Annual Antimicrobial Resistance Symposium

Poster Abstracts

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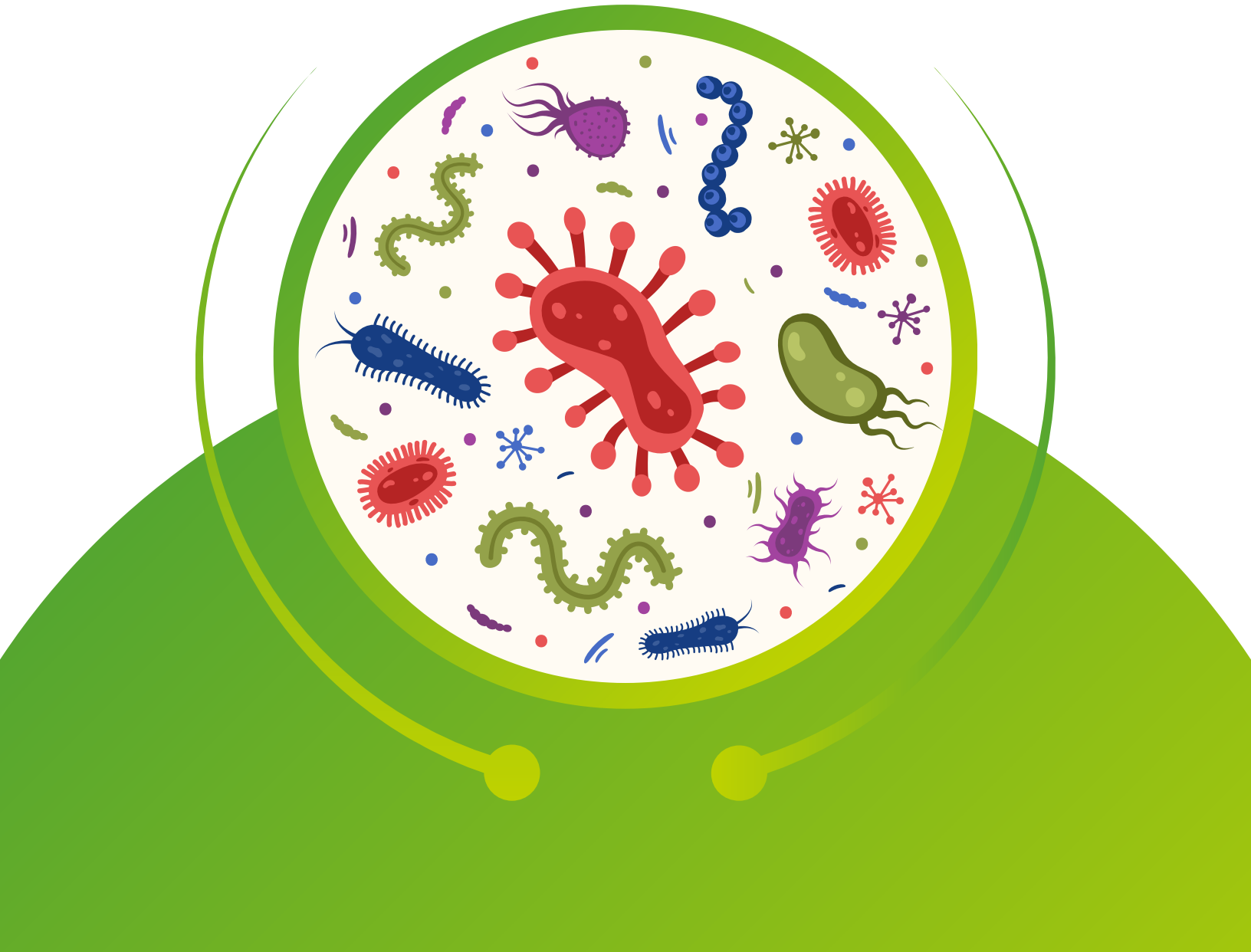

Poster Presentations

1. *Leveraging chemical biology approaches to identify and characterize novel compounds to combat Candida auris*

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Billions of people are infected by fungal pathogens every year. Candida species are the most frequent cause of systemic fungal infections in North America and the fourth most common cause of hospital-acquired infections. Candida auris is an emerging pathogenic yeast, which was discovered in 2009. It is now causing outbreaks around the globe and has been classified as a critical priority fungal pathogen by the World Health Organization. This is in part due to the fact that treatment of *C. auris* infections is complicated by the emergence of drug-resistant isolates and the limited arsenal of antifungal agents. Specifically, there are only three classes of antifungals to combat invasive disease: cell membrane targeting polyenes and azoles, and cell wall targeting echinocandins. Thus, novel therapeutic strategies are needed. The aim of my project is to leverage chemical biology approaches to discover compounds with activity against *C. auris*. To do so, I employed the Boston University Center for Molecular Discovery (BU-CMD) library consisting of 3,066 chemically diverse compounds to screen for molecules with activity against *C. auris*. This identified 44 hits, of which five were prioritized for further characterization. Current work has focused on evaluating the spectrum of activity of each molecule and employing genomic approaches to characterize the mechanism of action. Due to the broad spectrum of activity of CMLD012789 and CMLD012984 against diverse fungal pathogens, these compounds were explored for mode of action using haploinsufficiency profiling (HIP). HIP allows for the identification of putative cellular targets based on the principle that a genetic reduction in the gene that encodes the compound target will result in hypersensitivity to the compound. Future work will involve further characterization of CMLD012789 and CMLD012984 bioactivity, cytotoxicity against mammalian cells, and mode of action. Overall, this project has the potential to identify novel strategies to combat *C. auris* infections.

2. *A structure-guided approach to identify fungal-selective Yck2 inhibitors*

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Candida species are a major cause of invasive fungal infections, which result in ~1.5 million deaths annually. There is an urgent need to identify novel antifungal strategies, and targeting fungal kinases is a promising avenue. Yck2 is a fungal member of the casein kinase 1 (CK1) family and is important for Candida albicans virulence and cell wall homeostasis. A previous chemical screen revealed a 2,3-arylpyrazolopyridine, termed GW, as an inhibitor of Yck2, which results in fungal-selective growth impairment under physiological conditions and enhances antifungal efficacy. However, GW's poor metabolic stability presents a liability for its progression into in vivo studies. Therefore, we strived to optimize the GW scaffold through medicinal chemistry. Two sets of molecules derived from either the parent GW pyrazolo[1,5-a]pyridine core or an analogous imidazo[1,2-x]azine scaffold, were generated. Utilizing genetic and biochemical approaches, I characterized nine newly synthesized imidazo[1,2-x]azine derivatives. From these studies, two molecules, CTN1756 and CTN1844, were prioritized. Both demonstrated whole-cell bioactivity in a standard dose-response against C. albicans, and biochemical selectivity for the fungal Yck2 compared to human CK1α. Furthermore, CTN1756 and CTN1844 displayed on-target whole-cell activity, as compound treatment results in polarized growth of C. albicans, and enhanced

caspofungin efficacy in an echinocandin-resistant strain, consistent with Yck2 inhibition. Unfortunately, all imidazo[1,2-x]azine compounds tested to date exhibit low metabolic stability in vitro. Future work will focus on the generation of additional molecules to further optimize the potency, selectivity, and metabolic stability of the scaffold to optimize Yck2 inhibitors as a novel class of antifungal.

3. *Delivery of natural product antimicrobials to priority pathogens by Streptomyces extracellular vesicles*

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Majority of the clinical antimicrobials originate from *Streptomyces* specialized metabolites. We have found that *Streptomyces* often package their antimicrobial specialized metabolites into extracellular vesicles for secretion. The vesicles can accommodate antimicrobials with very different chemical properties, mechanisms of action, and spectrums of activity. These extracellular vesicles can serve as a drug delivery system. Using fluorescence transfer experiments, we show that the extracellular vesicles from three different *Streptomyces* strains can deliver molecular cargo to priority microbial pathogens. This includes *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans*, and most likely occurs by membrane fusion. This direct delivery of antimicrobials leads to increased antimicrobial efficacy, including against multidrug resistant strains. Understanding the delivery of antimicrobials and increased efficacy exhibited by these extracellular vesicles could help us develop improved therapeutics to counter antimicrobial resistance.

4. *Coagulase-negative staphylococci (CONS) positive blood cultures in the NICU: a quality improvement project*

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Coagulase negative staphylococci (CONS) can cause late onset sepsis in the NICU but can also be considered a contaminant in blood cultures resulting in unnecessary antibiotic use. As part of a quality improvement initiative in our NICU, we aimed to improve our treatment of CONS positive blood cultures. We implemented a standard approach for CONS positive cultures to improve contaminant diagnosis and reduce antibiotic use in Oct 2019. A before-after comparison of CONS positive cases (April-Sept 2019 compared to Oct 2019-Feb 2022) was completed using retrospective chart review. T-test, Fisher's exact test and Chi- squared tests were used to compare treatment outcomes pre and post algorithm and characteristics of cases deemed contaminant versus true infection as appropriate. Ninety-five CONS positive cases were reviewed, 82% of all isolates were cloxacillin resistant. Sixty-two cases were considered contaminants and 33 infections. After applying the algorithm, more cases of contamination post algorithm (n=57/78) compared to pre algorithm (n=7/17) were diagnosed (p=0.02). The mean time to positivity reduced significantly in the contaminant group after the algorithm was implemented (21.6 to 17.7h, p=0.03). We achieved 92% compliance with repeat cultures being collected before vancomycin was initiated, 100% compliance with stopping antibiotics when considered a contaminant and 75.9% compliance with treatment duration recommendations. The empiric antibiotic chosen was vancomycin in 24 cases (25.3%). Time to positivity, clinical status and repeat cultures can help to differentiate true infection versus contamination. In our analysis the mean time to positivity among infections was 15 hours, compared to a time to positivity of more than 24 hours in most studies being considered contamination. Providers diagnosed more contaminant cases after applying the algorithm. Compliance with all elements of the algorithm was more than 75%. Our QI initiative reduced the number of CONS positive cultures treated as infection. Repeating cultures prior to

starting vancomycin can assist in determining contaminated cultures. Future directions include further optimization of the algorithm (e.g., reducing the time to positivity cut-off to be deemed contaminant at 18hand reduce treatment durations to 5 to 7days if no line in situ) and ongoing improvements in blood culture collection and measurement of compliance to our algorithm to further reduce unnecessary antibiotic use in the NICU.

5. *Revolutionizing antifungal treatments: Unveiling synergistic actions between antifungals and mycoviruses*

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Infectious diseases remain a formidable global health challenge, causing substantial mortality and morbidity worldwide. Of particular concern is the escalating incidence of invasive fungal diseases, especially among immunocompromised populations. Antifungal resistance compounds these threats, necessitating innovative approaches to enhance treatment outcomes. In this study, we suggest exploring the synergistic interactions between antifungals and mycoviruses, viruses infecting fungi, to revolutionize fungal infection treatments. Mycoviruses, which exploit fungal vulnerabilities, hold promise in modulating fungal pathogenicity. By understanding how mycoviruses influence fungal virulence and metabolic pathways, targeted therapies disrupting essential fungal functions can be developed, enhancing susceptibility to drugs. Identifying hypervirulence hallmarks aids prognosis, guiding the development of combination therapies augmenting antifungal drug efficacy and mitigating resistance. Additionally, studying these interactions can shed light on antifungal resistance mechanisms, uncovering novel strategies to overcome antimicrobial resistance. We apply computational methods to find prospective candidate among mycoviruses to identify suitable models for exploring host-virus interactions. For many pathogens on WHO Fungal Priority Pathogen List, such models are limited or mostly absent. Preliminary work utilizing *Saccharomyces cerevisiae* and its viruses (L-A) as a model system offers deep insights, leveraging extensive genomic data for understanding intricate fungal-virus relationships. These insights can be extrapolated to less studied fungal organisms on the WHO's priority list, magnifying the impact of this research. In conclusion, investigating the synergies between antifungal drugs and mycoviruses represents an innovative avenue for combating fungal infections.

6. *Performance of two new MALDI ToF systems for identification of common and rare yeasts*

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During the last decade, MALDI ToF MS based identification of fungal pathogens improved steadily. New databases promise correct identification (ID) even of challenging species (e.g., *Magnusiomyces*, *Rhodotorula* and *Saccharomyces* spp. Our aim was to compare identification (ID) rates of the two most recent MALDI ToF MS systems, the VITEK® MS PRIME bioMérieux) and MALDI Biotyper® sirius (Bruker Daltonics), using a collection of common and rare species.

7. *Identification and characterization of molecules with novel antifungal activity against Candida albicans*

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Infection with Candida albicans, one of the most prevalent fungal pathogens of humans, causes a diverse range of diseases extending from superficial infections to deadly systemic mycoses. Currently, only three major classes of antifungal drugs are available to treat systemic infections: azoles, polyenes, and echinocandins. Alarmingly, the efficacy of these antifungals against *C. albicans* is hindered both by basal tolerance towards the drug and the development of resistance mechanisms such as alterations of the drug's target, modulation of stress responses, and overexpression of efflux pumps. My research focuses on the identification and characterization of compounds with novel activity against *C. albicans*. Leveraging the Boston University Center for Molecular Discovery (BU-CMD)'s chemical library, I screened 3,280 compounds against a *C. albicans* clinical isolate and identified 16 molecules that inhibit *C. albicans* growth through metal chelation. Media supplementation with ferric or ferrous iron rescued *C. albicans* growth, indicating these compounds exert their antifungal activity primarily through iron chelation. Furthermore, I characterized the mode of action of two compounds with novel antifungal activity from the Broad Institute Diversity-Oriented Synthesis (DOS) library. Through genetic approaches, I identified one molecule as an inhibitor of Erg11, despite the compound's lack of a canonical azole ring, and another compound as a translation inhibitor. Future work will leverage biochemical methods to further define compound mode of action and focus on investigating the therapeutic potential of prioritized molecules. Overall, my research has identified compounds with novel antifungal activity that may have potential for much-needed, future drug development.

8. *Inhibition of the Salmonella novel E3 ligase, SspH1, by engineered ubiquitin variants*

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A key component of the pathogenesis of gram-negative bacteria is the secretion of effector proteins through a type III secretion system (T3SS). One family of effectors secreted through T3SS are Novel E3 ligases (NELs). NELs are known to mediate the transfer of ubiquitin onto host proteins which can impact their function, alter their localization, or direct them for degradation by the proteosome. NELs are increasingly becoming relevant in the study of bacterial pathogenesis but inhibitors specific to NEL activity are underdeveloped and uncharacterized. Our research focuses specifically on Salmonella secreted protein H1 (SspH1), which impacts PKN1 stability and downregulates the pro-inflammatory response during Salmonella infection. To address the lack of NEL inhibitors we pursued the use of engineered ubiquitin variants (Ubvs) since it is known that NELs directly interact with ubiquitin. Through this pursuit we have identified two Ubvs, A06 and D09, with an increased binding affinity for SspH1 when compared to wildtype ubiquitin. We explored the consequences of co-expressing these Ubvs alongside SspH1, or the catalytically mutant SspH1C492A, within eukaryotes using Saccharomyces cerevisiae as a model system. Analysis of *S. cerevisiae* co-expressing SspH1 or SspH1C492A and Ubv A06 or D09 by spot assay and growth curve analysis revealed SspH1-mediated growth interference which occurred in a catalytically dependent manner. Co-expression of either Ubv A06 or D09 was sufficient to prevent SspH1-mediated interference in *S. cerevisiae* while also having no effect on *S. cerevisiae* growth

when expressed alone or with SspH1C492A. We further characterized the SspH1-Ubv interaction through in vitro ubiquitination assays to directly explore the effect of Ubvs on the ubiquitination process. Analysis of SspH1 activity in the presence or absence of Ubv D09 revealed significant alterations to SspH1-mediated ubiquitination. We were also able to determine that Ubv D09 significantly altered the ubiquitination of the known SspH1 substrate, PKN1.

9. *Antibiotic resistance and serotype distribution of Streptococcus pneumoniae in Toronto and Peel regions of Ontario, Canada*

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[1]Department of Health Research Methods, Evidence and Impact, McMaster University; Department of Microbiology, [2]Mount Sinai Hospital

To investigate changes in the distribution and antimicrobial susceptibility of *Streptococcus pneumoniae* isolates causing invasive pneumococcal disease (IPD) among patients with IPD in Metropolitan Toronto and Peel regions in Ontario, Canada, between 2014 and 2022. And also, to assess the temporal pattern of *S. Pneumoniae* serotype distribution and replacement in relation to those targeted by vaccines during this period. Between January 02, 2014, to December 31, 2022, the Toronto Invasive Bacterial Diseases Surveillance Network (TIBDN) analyzed 2,194 S. pneumoniae isolates from IPD infections in Toronto and the metropolitan region of Peel. The broth microdilution method was used for antimicrobial susceptibility testing and interpretation was done using the CLSI standards. Pneumococcal antibodies from the Statens Serum Institute in Copenhagen and the Quellung reaction were used to identify serotypes. Of the 2,194 eligible isolates, 1057 (48.17%) were from adults 15 – 65 years old, 866 (39.47%) from adults >65 years old, and 271 (12.35%) from children 0-15 years old. Isolates were mostly recovered from blood (2062; 93.98%). There were 67 (3.05%) cerebrospinal fluid (CSF) and 48 (2.18%) pleural fluid specimens. Other specimens included sputum, bronchoalveolar lavage, synovial/joint fluids, peritoneal fluids, wounds, abscesses, and aspirates from various tissues. Serotyping was performed on 2039 isolates. Serotypes 3 (242/11.86%); 22F (191/9.37%); 19A (158/7.75%); 23A (117/ 5.7%), 15A (92/4.51%), 11A (85/4.12%), 9V (84/4.12%) and 23B (70/3.4%) were the most prevalent. There were five truly not typable isolates. Antibiotic susceptibilities were not available for 134 (6.11%) isolates. Isolates were assessed for their resistance to penicillin, amoxicillin, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, and erythromycin. Data analysis on changes in isolates' distribution, antibiotic susceptibility profile, and the temporal pattern of the serotype distribution of 2194 isolates from IPD infections in the Toronto and Peel region between 2014 and 2022 is being conducted; final results will be presented.

10. *Quantifying the sheltering effect of β-lactamase production by resistant bacteria on bacteria that are susceptible to β-lactam antibiotics*

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β-lactams are the most used class of antibiotics worldwide. Unfortunately, their clinical utility is severely threatened by the spread of resistant bacteria that produce β-lactamases, enzymes that inactivate β-lactams. The production of β-lactamases by resistant bacteria can "protect" bacteria that would otherwise be susceptible to β-lactams; this has been described as a sheltering effect. Antibiotic sheltering can complicate the clinical treatment of infections and can even contribute to β-lactam treatment failure, especially in polymicrobial infections. We developed a luminescence reporter system that directly measures bacterial growth and can be applied to quantify the protective effect of sheltering. We demonstrate that the level of sheltering depends on both the type of β-lactamase and the antibiotic that is used. Using this approach, we observed that

carbapenemase-producing *Escherichia coli* can shelter susceptible *E. coli* cells from carbapenem treatment at concentrations that are 128 times greater than the lethal concentration. We also show how antibiotic sheltering is impacted by factors that alter the cell wall of β-lactamase-producing bacteria, including the disruption of genes encoding for porins and other outer membrane proteins. We also demonstrated that strains which produce higher levels of outer membrane vesicles (OMVs) are associated with even higher levels of sheltering. In contrast, disruption of the porins that are normally used for β-lactam entry led to decreases in the levels of sheltering. Future work will be aimed at characterizing other factors that contribute to sheltering, such as exposure to other classes of antibiotics, and microbial metabolites.

11. *VITEK2 advanced expert system β-Lactam resistance phenotyping compared to whole genome sequencing in Enterobacterales isolates from European medical centres*

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The rapid detection of β-lactam resistant phenotypes such as transferable AmpC (tAmpC), ESBL, and carbapenemase are important for appropriate antimicrobial therapy administration and infection control.

The VITEK2 Advanced Expert System (AES) provides interpretations of β-lactam resistance phenotypes based on an extensive database of MIC distributions and prevalent resistance mechanisms in Enterobacterales isolates.

In this study, the AES β-lactam resistance phenotypes were compared to whole genome sequencing results from 572 European Enterobacterales isolates.

12*. Investigating the occurrence of antimicrobial resistance in the environment in Canadian settings: a scoping review*

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Antimicrobial resistance (AMR) is an environmental, agricultural, and public health problem that is impacting the health of humans and animals. The role of the environment as a source of and transmission pathway for antimicrobial resistant bacteria (ARB) and antimicrobial resistant genes (ARGs) is a topic of increasing interest that to date has received limited attention. This study aimed to identify the sources and pathways contributing to ARB and ARGs dissemination through bioaerosols (air and dust), water, and soil in Canada using a scoping review methodology and systems thinking approached. In the system map, we represent the occurrence and relationships between sources and pathways for AMR dissemination through water, soil, and bioaerosols. This figure guided development of the scoping review protocol, specifically the keywords searched and how and what data were extracted from the included studies. In total 102 studies of AMR in water, 67 in soil, and 12 in air were identified from Canada. Studies to detect the presence of ARGs have mainly been done in wastewater treatment plants and concentrated animal feeding operations. We also identified several points that need to be investigated in the systems map to have a better understanding of AMR dissemination between different elements of the environment. The data extracted from these studies will be used to parameterize environmental transmission models for AMR. Specifically, additional pathways will be added to an existing integrated assessment model that investigates the relative exposure to AMR in humans and through different animal production chains. The results of this study will help identify points of intervention for AMR mitigation, provide

evidence for public health policy implementation to help minimize exposure to AMR through the environment and determine key areas for future research.

13. *Leveraging a structure-guided approach to explore the potential of targeting Cryptococcus Hsp90*

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Fungal pathogens have an enormous impact on human health worldwide. In particular, *C. neoformans* is one of the most detrimental fungal pathogens to human health. Despite this concerning trend, only the azole and polyene classes of antifungal drugs can treat cryptococcal disease. Thus, there is a clear need to develop novel antifungals to treat *C. neoformans* infections. A promising antifungal target is the molecular chaperone Hsp90. Despite its important role in enabling virulence and drug resistance in diverse fungal pathogens, in the setting of infection, the inherent toxicity of inhibiting host Hsp90 necessitates the development of fungal-selective compounds. To explore targeting Hsp90 as a strategy to combat *C. neoformans* infections, this work leverages a structure-guided approach to develop and characterize fungal-selective Hsp90 inhibitors. Specifically, 10 novel Hsp90 inhibitors were synthesized based on a resorcylate aminopyrazole (RAP) scaffold and tested for fungal selectivity, potency, and whole-cell activity. A fluorescence polarization (FP) assay was used to determine how potent and selective the compounds were towards Cryptococcus and human Hsp90 from cellular lysates. Results from this assay indicated that seven of the ten compounds were highly potent against fungal Hsp90 (EC50<25 μM) with moderate activity against human Hsp90 (EC50 > 25 μM), resulting in excellent selectivity. Next, a dose-response assay was used to determine if these compounds had whole cell activity against *C. neoformans*. Unfortunately, none of the compounds were efficacious against wild type *C. neoformans* (MIC80 > 25 μM), highlighting additional work needs to be done to develop fungal-selective compounds with activity against fungal cells. Overall, this work aims to uncover new therapeutic strategy.

14. *CRISPR-based diagnostics for Candida detection and identification of antimicrobial resistance mutations*

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Antimicrobial resistance (AMR) is a growing global issue, impacting over 1 billion people worldwide who have suffered from fungal infections. Of particular concern is the emergence of multi-drug resistant Candida species, notably Candida auris, as it is recognized on WHO's fungal pathogen priority list due to its high transmission rates and potential to cause deadly hospital outbreaks. It is therefore imperative that we dedicate our efforts to advancing new technologies that will enhance AMR surveillance, management of outbreaks, and antimicrobial stewardship. Current techniques that monitor AMR require multiple rounds of culture and analysis in centralized reference laboratories, causing delays in diagnosis and personalized treatment. To overcome these challenges, we propose a highly sensitive and specific CRISPR-based diagnostic for the detection of genetic biomarkers associated with antifungal resistance in Candida species. CRISPR-based diagnostics offer numerous advantages including rapid, isothermal, and cost-effective results that can be tested directly at the point of care. Here, we will present several CRISPR assay designs and investigate their effectiveness in identifying specific targets within Candida species, as well as

possible resistance mutations associated with azole and echinocandin antifungal agents. This research will demonstrate a novel approach for rapidly analyzing clinical samples and establish a foundation for future endeavours aimed toward improving AMR and infectious disease diagnostics.

15. *Elucidating mechanisms of susceptibility and resistance to distinct microsporidia species in wild isolates of C. elegans*

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Microsporidia are rapidly emerging opportunistic infectious pathogens that infect humans and most other organisms including agriculturally important species such as honeybees and fish[1,2]. Several parasitic microsporidian species have been isolated in wild strains of *C. elegans*[3]. Using PhenoMIP, a multiplexed sequencing-based screen for measuring phenotypes in *C. elegans*, 22 wild strains were tested against four microsporidian species. We identified two strains, JU1400 and MY1, which are sensitive to an epidermal-infecting species, yet resistant to an intestinal-infecting species. Complementation tests between JU1400 and MY1 suggests these strains share variants in the same genes that are responsible for sensitivity and resistance. Using genetic mapping, we identified four distinct loci which may be responsible for these differential phenotypes. Generation of introgressed lines narrowed critical region of the sensitivity phenotype to 700kb on the left arm of Chromosome I[4]. I plan to test genes with shared variants in MY1 and JU1400 to identify the causative genes responsible for their susceptibility phenotype. Concurrently, I plan on performing a forward genetic screen to identify genes responsible for the resistance phenotypes. Finally, I will expand the PhenoMIP assay to examine a greater number of wild isolates to identify additional strains with variations in fitness. With a larger panel of wild strains, causative variants can be identified through genome-wide-association studies. Overall, we will identify key molecular mechanisms in host-pathogen interactions between *C. elegans* and microsporidia that serves as a model to understand these types of infections in humans.

16. *Verification of antimicrobial susceptibility testing methods for new agents against challenging multi-drug resistant Gram-negative organisms*

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The rise of antimicrobial-resistant bacteria is a rapidly growing threat, particularly among multi-drug resistant Gram-negative (MDR GN) organisms. Timely completion of antimicrobial susceptibility testing (AST) is therefore critical, and laboratories often rely on commercial automated AST methods, rather than the more time-consuming gold-standard, frozen broth microdilution (BMD). Newer drugs are often not included in these automated ASTs, forcing laboratories to turn to alternate AST methods that may not have been well-verified. Previously we verified different methods to test newer agents (ceftobiprole, cefiderocol, ceftolozane/tazobactam, ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam, plazomicin, and tigecycline). The purpose of this study was to compare these methods against one another. A custom frozen BMD Sensititre panel (ThermoFisher Scientific) served as gold-standard. Verification data for a custom lyophilized BMD Sensititre panel (ThermoFisher Scientific), Etest gradient strips (bioMérieux), MIC test strips (Liofilmchem), and Kirby-Bauer disks (Liofilchem) were compared. A total of 90 Enterobacterales, 46 Pseudomonas aeruginosa, 39 Acinetobacter spp., and 15 non-lactose fermenters were tested. Very major errors, major errors, minor errors, and categorical/essential agreements were calculated, and acceptability was determined using both

CLSI thresholds and 95% confidence intervals. Where applicable, acceptability was recalculated using the CLSI-endorsed error-rate bound method. Heat maps of acceptability were created to facilitate between method comparisons. For Enterobacterales, all methods are acceptable for newer agents, except tigecycline. For *P. aeruginosa*, *Acinetobacter* spp., and non-lactose fermenters, the lyophilized BMD Sensititre performed the best and is acceptable for all newer agents, except tigecycline (*Acinetobacter* spp. and non-lactose fermenters), meropenem/vaborbactam (*P. aeruginosa*), and ceftobiprole (*Acinetobacter* spp.). Testing new antimicrobial agents that are included in automated susceptibility testing platforms poses challenges to laboratories. Not all alternate methods have acceptable performance compared to gold standard BMD testing. Laboratories should take this into consideration when choosing their testing methodologies and should complete verification of the accuracy of their chosen testing method.

17. *Continued reduction in the frequency of carbapenemase-producing Enterobacterales during COVID-19*

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Carbapenemase-producing Gram-negative Enterobacterales (CPE) are an increasing threat. Introduction of CPE is facilitated by international travel from areas of high prevalence to regions of low prevalence. International travel was dramatically reduced in 2020 and 2021 due to the COVID-19 pandemic. Correspondingly, CPE numbers in a tertiary-care clinical microbiology laboratory servicing a metropolitan urban region decreased during this time period. The goal of this study was to review changes in the frequency of CPE at this site in 2022, given an increasing amount of global flights from 2021 to 2022. The total number of CPE per year (all CPE per year excluding duplicates) and the total number of individual carbapenemases per year (all carbapenemase per year excluding duplicates) were graphed from 2009 through 2022. The trend from 2009 through 2019 (pre-COVID-19) was compared to that from 2019 through 2022 (COVID-19). Chi-squared test for trend was completed for each time period using GraphPad Instat. 244 CPE were identified from 2009-2022. Despite the rise in global flights from 2021-2022, there was a continued reduction in the number of CPE. From 2019 through 2022, CPE isolates declined from 29 to 22 to 20 to 16. The frequency of CPE correlated with lower amounts of all detected carbapenemases, with the exception of KPC enzymes. Despite a rise in international travel, the frequency of CPE has continued to decline in 2022. However, the rise in KPC enzymes may signify more travel between regions with greater prevalence of this enzyme or higher endemic prevalence of this enzyme.

18. *A reduction in Candida parapsilosis susceptibility to fluconazole between 2019 and 2021*

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Antimicrobial resistance continues to be a concern for bacterial and fungal species. An academic hospital microbiology laboratory in a metropolitan urban region was recently alerted to concerns of rising numbers of fluconazole-non-susceptible *Candida parapsilosis* in an ICU. The aim of this work was to compare *C. parapsilosis* susceptibility to fluconazole over time across multiple hospitals served by the academic hospital laboratory. Consecutive *C. parapsilosis* blood isolates from four tertiary-care hospitals, one per patient per year, from 2016 through 2021 were reviewed. The proportion of isolates that were fluconazole susceptible (defined as susceptible or susceptible dose-dependent) were graphed with 95% confidence intervals calculated using GraphPad

QuickCalcs. Trends were compared across sites. A total of 213 *C. parapsilosis* blood isolates were identified from four hospitals (range 20-105/hospital) from 2016 through 2021. A reduction in fluconazole susceptibility was noted in all hospitals from 2019 to 2020 to 2021 from a weighted average percent susceptibility of 100% to 85% to 76%, respectively. Among the index ICU ward, percent susceptibility to fluconazole among blood *C. parapsilosis* isolates dropped from 100% to 58% to 29% over the same time period. A reduction in fluconazole susceptibility in *C. parapsilosis* was noted across four different hospital sites with a dramatic drop noted in susceptibility in ICU isolates at one hospital between 2019 and 2021. Molecular typing and epidemiological analysis are ongoing to determine relatedness of isolates and possible nosocomial transmission. Trending data over time and by hospital ward in real-time would have allowed an earlier detection of this concerning reduction in fluconazole susceptibility in *C. parapsilosis*.

19. *Perceived ease-of-use and operator performance of a rapid multiplex PCR diagnostic system in a near-patient setting*

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Access to multiplex molecular diagnostic tests for the rapid and accurate diagnosis of respiratory tract infections is limited in the near patient setting but has potential to improve patient outcomes and antibiotic stewardship. The BIOFIRE® SPOTFIRE® Respiratory(R) Panel (bioMérieux, SaltLakeCity, UT), designed for use with the BIOFIRE® SPOTFIRE® System is an Investigational Use Only (IUO) PCR-based sample-to-answer diagnostic test that identifies four bacteria and 11 viruses from nasopharyngeal swabs (NPS) in ~15minutes. This study evaluated the ease-of use and operator performance of the IUO SPOTFIRE® R Panel in the near-patient setting.

20. *An analysis of the value added of subgroup stratification of antibiograms*

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The Infectious Disease Society of America and Clinical and Laboratory Standards Institute recommend the use of stratified antibiograms to guide empiric clinical treatment. Generating annual unit- and specimen-specific susceptibility reports is a laborious task involving large datasets and data manipulation. This stratification is often limited to academic hospital-based laboratories due to resource allocation challenges. This study compares differences in antibiotic susceptibility between an accumulative hospital-wide, all-specimens antibiogram and stratified antibiograms in order to identify the value added of subgroup stratification of antibiograms. Antibiotic susceptibility of bacterial isolates from 2021 at a tertiary-care academic hospital was obtained from published accumulative and unit- and specimen-specific stratified antibiograms. Differences in percent susceptibility by organism and drug between the accumulative and stratified antibiograms were calculated. A weighted proportion antibiogram percent susceptibility (WPAPS) was calculated for the accumulative and each stratified antibiogram. Heat maps representing differences in WPAPS were created and differences in WPAPS were compared for significance using Chi-squared Test. Excel and R Statistical Software were used for graph generation. GraphPad QuickCalcs was used for statistics. Antibiograms from emergency department (ED) samples had significantly higher susceptibility as measured by WPAPS whereas intensive care unit (ICU) and transplant (TR) show reduced susceptibility. Blood isolates and urine isolates were significantly less susceptible

compared to hospital-wide isolates. In subgroup analysis, bloods samples showed significantly higher susceptibility in the ED and significantly reduced susceptibility in the ICU. Urine shows higher susceptibility in ED and lower susceptibility in TR. Respiratory samples in TR showed a large reduction in susceptibility. There are unit- and specimen-specific differences in susceptibility compared to accumulative hospital-wide antibiogram susceptibilities suggesting the need for unit- and specimen-specific antibiograms for optimal empiric antimicrobial management.

21. *Antibiograms: Value of trend analysis*

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Annual antibiograms serve as a guide for empirical therapy. However, susceptibility is presented as an average percent susceptible, providing only a single static interpretation of susceptibility. To detect emerging antimicrobial resistance, especially those that are subtle, graphing susceptibility over time can visualize trends. The purpose of this study was to analyze susceptibility trends in the most prevalent organisms in a single tertiary-care academic hospital over time. Antibiotic susceptibility of bacterial isolates of blood and urine samples from 2013 to 2021 of a tertiary-care academic hospital was obtained from annual antibiograms created following the Clinical and Laboratory Standards Institute (CLSI) M39 document. Updated CLSI M100 breakpoints were used for all antibiograms throughout the study period. Trends were shown using Excel. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the most prevalent Gram-negative organisms from blood and urine cultures. *E coli* displayed a subtle trend in decreasing susceptibility for ceftriaxone, and a similar trend is seen for ceftriaxone, trimethoprim-sulfamethoxazole, and tobramycin in *K. pneumoniae*. From 2019, urine isolates show a notable reduction of 38% in tobramycin susceptibility in *E. coli* and 78% reduction in *K. pneumoniae*. Not shown in Figure 1, blood, and urine *P. aeruginosa* and blood and urine *Staphylococcus aureus* and enterococci, the most prevalent Gram-positive organisms, showed relative stability in susceptibility. Reporting antimicrobial susceptibility over time for bacterial isolates, as opposed to only reviewing static data at a single point of time, is valuable for detecting emerging resistance. Subtle trends, such as those seen for ceftriaxone from blood Gram-negative isolates, and dramatic trends, such as those seen with tobramycin from urine Gram-negative isolates, can go unnoticed by review of mass data sets presented in separate disconnected annual antibiograms.

22. *Reduced susceptibility in Candida species: value of Candida antibiograms and trend analysis*

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The emergence of antimicrobial-resistant organisms poses an ongoing challenge. Emerging resistance is largely focused on bacteria with hospital data presented typically in single-time-point formats. Reduced fluconazole susceptibility in *Candida parapsilosis* was recently identified as a concern by antimicrobial stewardship at an urban academic hospital. This study investigates the value in creating antibiograms for all of the most commonly isolated *Candida* species and evaluating their susceptibility trends over time. Antifungal susceptibility of *Candida* spp. isolated from blood cultures between 2013 to 2021 from four academic hospitals served by the same clinical microbiology laboratory was analyzed using Excel. Susceptibility testing and result interpretations followed 2022 Clinical and Laboratory Standards Institute (CLSI) M100 guidance. Reported results reflect the first isolate of a given species per patient per year following the CLSI M39 document, with the exception of *C. albicans* from 2020 and 2021 where testing was selective due to global supply shortages. Susceptibility data for the six most commonly isolated *Candida*

spp. were analyzed. *C. albicans* and *C. glabrata* showed relative stability in susceptibility over time. Stable trends in all antifungals were noted for all other species with the exceptions of fluconazole and capsofungin. *C. parapsilosis* showed concerning decreases in susceptibility for both 400 and 800mg/d fluconazole dosing in 2020 and 2021. This is also seen in recent years for *C. tropicalis*, and to a lesser extent for fluconazole 400 mg/d susceptibility in *C. dubliniensis*. *C. krusei* had a concerning decrease in capsofungin susceptibility in recent years, not seen in other species. Analyses of *Candida* species susceptibility is valuable to inform empiric antifungal choice and should be included along with bacterial data on annual antibiograms. Reporting susceptibility data over time is instrumental in detecting emerging trends that cannot be easily deduced in a standalone single-point-in-time antibiogram.

23. *A rapid luminescence-based diagnostic assay for the detection of carbapenemase-producing organisms*

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Infections caused by carbapenemase-producing organisms (CPOs) pose an increasingly urgent global health threat due to the ability of carbapenemases to degrade carbapenems, a group of last resort antibiotics. Due to the poor patient outcomes and high mortality rates that are associated with infections caused by CPOs, early detection of these pathogens is vital to ensure optimal antimicrobial therapy is administered in a timely manner. Currently, the gold standard assays for carbapenemase detection are based on assessing microbial growth in the presence of a carbapenem (e.g., the modified carbapenem inactivation method, mCIM). Although these assays are widely used due to their ease of use and relatively high sensitivity, they suffer from long turn around times, which can delay the initiation of appropriate antibiotic therapy. Although rapid assays that measure carbapenemase activity have been developed, they employ expensive substrates (e.g., nitrocefin) or suffer from low sensitivity with applied to the detection of certain weak carbapenemases, such as OXA-type enzymes (e.g., CARBA-NP test). Thus, there is a need for new rapid detection assays that can reduce turn around time without requiring costly reagents or sacrificing sensitivity. We report the development of a luminescence-based biosensor that can be applied to the detection of CPOs. This proof-of-principle study demonstrates that our method can reliably detect OXA-48, KPC-2, IMP-1, and NDM-1-producing *Escherichia coli* within 90 minutes with a high degree of sensitivity and specificity. Additionally, β-lactamase inhibitors can easily be introduced into the workflow to provide additional information on the type of carbapenemase being produced. This assay is compatible with samples obtained from both plate and liquid cultures, does not require the use of expensive substrates, and involves minimal preparation time.