Tuberculosis and Innovative Diagnostic Approach

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Tuberculosis (TB) is a global public health concern, killing an estimated 1.5 million people worldwide in 2020 alone, necessitating prompt and accurate diagnostics for better outcomes. There are several existing diagnostic tests for TB including skin tests, patient sputum samples, and blood tests. These diagnostic tools are difficult to implement when it comes to isolated areas and in patients who cannot produce sputum such as children and the elderly. Urine is a desirable biofluid for diagnostics because it can be collected noninvasively in abundant amounts. We designed a unique collection device for urine to permit subsequent molecular analysis. TB markers exist in urine at a very low concentration, and transportation of urine to the pathological labs can be an issue, as it requires costly refrigeration. Our collection device addresses these two concerns. A biomaterial that captures, concentrates and preserves bio-molecules is incorporated in the collection device. Using this affinity material, we can detect mycobacterium tuberculosis molecules in the urine of TB patients; mass spectrometry and PCR enabled sensitive detection of protein, glycolipid, and DNA derived from TB in urine. Additionally, the affinity net was used sequester extracellular vesicles (EVs) from TB patients, which also contain TB antigens. Finally, our origami collection cup was acceptable to patients based on standardization surveys conducted in Nepal and Guinea Bissau. In conclusion, the collection cup is the novel tool for TB diagnostics which allows for the isolation and characterization of TB antigens and EVs from infected patient's urine.

Characterization of a Novel, Fastidious, Gram-negative Bacilli Isolated from Clinical Wound Specimens

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Background: Epidemiology studies have shown a rise in dog bites during the COVID-19 pandemic. In 2020, we received a facultatively anaerobic, non-hemolytic Gram-negative rod (OL1) from a dog bite wound for identification. 16S rRNA gene sequencing showed OL1 was 95.9% identical to *Ottowia pentelensis* in the family *Comamonadaceae*. Our historical sequence database revealed 8 additional isolates (OL2-OL9) from hand wounds/abscesses (including 3 dog bites) since 2012 that had ⩾99.8% identity with OL1. Most other *Ottowia* sp. have been isolated from industrial and food sources, with no reports from patient samples. As these clinical isolates likely represent a novel *Ottowia* species, we aimed to characterize them using both phenotypic and genomic approaches.

Methods: The OL isolates were tested in API20NE panels. Paired-end genomic DNA libraries were sequenced as 150nt reads by Illumina NovaSeq. *De novo* assembly, functional prediction, and phylogenetic analyses were performed.

Results: All 9 OL isolates were negative for indole, urea, arginine, esculin, PNPG, glucose fermentation and carbohydrate assimilation tests. Potassium gluconate assimilation and gelatin hydrolysis were positive for 5 and 4 isolates, respectively. The estimated genome size was ~3.1 Mbp, with 66.1% G/C, and ~3523 genes. Potential virulence factors, multidrug efflux systems, and 1-2 intact prophages were identified. Genomic phylogenetic analysis showed the OL isolates clustered separately from all known *Ottowia* spp.

Conclusions: These OL isolates are fastidious, Gram-negative bacilli from clinical wound specimens, and are associated with dog bites. Genomic and 16S rRNA gene sequence analysis suggests these isolates constitute a novel species within the family *Comamonadaceae*.

Key words: *Ottowia*, phenotypic analysis, Whole-Genome Sequencing

Cellvibrio japonicus degradation of fungal necromass activates Carbohydrate Active Enzyme-encoding genes in a substrate-specific manner

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Microorganisms are often the drivers of decomposition and therefore have important roles in global nutrient cycling. In particular, the degradation of fungal biomass (necromass) by other fungi and bacteria is proving to be a key contributor to soil health. However, the mechanisms of necromass degradation are understudied. To help address this knowledge gap, we conducted a multi-timepoint transcriptomic analysis of the Gram-negative bacterium *Cellvibrio japonicus*, which has been previously identified as a necromass decomposer *in situ*. Our analysis included differential gene expression comparisons using the complete necromass of *Meliniomyces bicolor*, which exists in high and low melanin forms. The highly melanized *M. bicolor* possess another level of cell wall recalcitrance due to the complexity and insolubility of melanin. Our results found *C. japonicus* growth and necromass decomposition was overall stronger on the low melanin necromass compared to high melanin. As expected, gene expression analysis resulted in up-regulation of numerous carbohydrate active enzymes (CAZymes) with different functions on each necromass type. Furthermore, we observed a dynamic change in gene expression between exponential growth and stationary phase on each necromass type which appeared to be substrate dependent. Specifically, this comparison exposed a shift in CAZyme up-regulation on necromass, indicative of the stepwise decomposition of *M. bicolor*. Overall, these transcriptomic studies provide the first steps towards assessing the physiological relevance of up-regulated CAZymes and provide the foundational data to generate a comprehensive and predictive model of necromass decomposition by soil bacteria.

Prevalence of Antimicrobial Resistance in *E. coli* Isolated from Environmental Samples in Central Virginia

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Antimicrobial resistance (AMR) is a public health threat predicted to cause 10 million deaths annually by 2050. Recognizing the importance of AMR in our environment, this study aims to assess the prevalence of AMR in *E. coli* isolated from environmental samples, including livestock, waterfowls, wastewater treatment facilities, and drainage areas of different land use systems (crop, forest, grass, and urban land). A total of 313 *E. coli* isolates were obtained from the environmental samples in Central Virginia between 2020 and 2022. They were tested for their susceptibility to 12 antimicrobial agents approved by the US Food and Drug Administration for clinical use. Approximately 2.2% of the tested *E. coli* were resistant to five antimicrobials, with 3.7% multidrug resistance. Of the isolates, 75% isolates were resistant to at least one antimicrobial agent. All isolates were susceptible only to meropenem and chloramphenicol. The findings of this study demonstrate the risk of environments as a natural habitat for AMR *E. coli.* Continued research efforts on a larger scale are needed to determine the cause(s) of the observed prevalence of AMR in the bacteria in relation to the environment and their genomic relatedness.

In Vitro Antibacterial Activity of Nimbolide Against *Helicobacter pylori*

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Due to the rise in antibiotic resistance rates for the gastric pathogen *Helicobacter pylori,* there is a need to develop novel therapeutics to treat chronic H. pylori infections. We previously found that an extract of neem oil from the neem tree (*Azadirachta indica A. Juss*) displayed potent in vitro antimicrobial activity against *H. pylori* – compelling data given that the antimicrobial properties of *A. indica* components have been employed in agriculture and the food industry for many years. However, given the complex nature of neem oil, we next sought to define the specific compound responsible for this activity against *H. pylori*. To this end, we tested the antimicrobial activities of commercially available components of neem: azadirachtin, gedunin, and nimbolide. We found that only nimbolide was highly active against *H. pylori*. The bactericidal activity was time- and dose-dependent, independent of active H. pylori growth, and synergistic with low pH. Importantly, nimbolide-mediated cell death was irreversible after exposure to high concentrations. Excitingly, both neem and nimbolide were more effective against killing *H. pylori* biofilms than amoxicillin, which is part of the *H. pylori* treatment regimen. Overall, nimbolide had significant bactericidal activity against *H. pylori*, killing both free living bacterial cells as well as cells within a biofilm. Furthermore, a lack of hemolytic activity, synergistic activity at low pH, and bactericidal properties against bacteria in a state of growth arrest are all ideal pharmacological and biologically relevant properties for a potential new agent. Future studies will investigate the in vivo effectiveness of nimbolide.

Pseudomonas aeruginosa Increases Antimicrobial Production in Response to *Staphylococcus aureus*

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Bacteria often exist in multispecies communities where cooperative and competitive interactions affect community composition and physiology. It is common for bacteria to sense other microbes and respond antagonistically in a phenomenon known as competition sensing. Cystic fibrosis is a life-threatening condition in which persistent respiratory polymicrobial infections are common, and the interactions between the bacterial pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus* exacerbate disease severity. Since *P. aeruginosa* frequently resides in multispecies environments and produces a plethora of antimicrobials, we hypothesized that *P. aeruginosa* senses *S. aureus*, and subsequently responds by producing antimicrobials that are active against *S. aureus*. Thus, we measured the potency of *P. aeruginosa* supernatant grown in the presence or absence of *S. aureus* exoproducts. Supernatant from *P. aeruginosa* exposed to *S. aureus* secreted products significantly decreased *S. aureus* growth compared to the control, signifying that *P. aeruginosa* participates in competition sensing of *S. aureus*. To identify the antimicrobials induced by competition sensing, we measured specific anti-staphylococcal molecules in the supernatants and found that rhamnolipid surfactants were increased nearly two-fold. Additionally, expression of biosynthesis genes for rhamnolipids and the siderophore pyoverdine were significantly upregulated. Testing of molecules previously implicated in the sensing of *S. aureus* showed that the *S. aureus* amphipathic peptides phenol-soluble modulins (PSMs) induce promoters of these biosynthesis genes. Additionally, exogenous detergents also induce these promoters, suggesting that sensing of biotic surfactant stress drives the competitive response. Overall, these results define a competition sensing phenomenon where *P. aeruginosa* senses *S. aureus* PSMs and increases the production of antimicrobials including rhamnolipids.