Early Career Talks

Performance of a Qualitative Real-Time RT-PCR Assay for Measles Virus

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Background: Accurate, rapid measles testing informs and guides public health efforts, making a national reference laboratory offering beneficial. This study describes the performance of a RT-PCR laboratory developed test for this re-emerging pathogen.

Methods: Throat and nasopharyngeal (NP) specimens were collected with eSwabs or swabs transported in VCM, UTM, or M4 media. RNA was extracted and amplified with reverse transcription targeting a 75-base pair sequence conserved within the measles nucleoprotein (N) gene. Analytical parameters evaluated included sensitivity, specificity, stability, reproducibility, accuracy, and a cross-site study with another CLIA-accredited laboratory.

Results: Analytical sensitivity testing attained a low limit of detection (LoD) of 6-57 copies/mL. Both in vitro and in silico analyses yielded no observed cross-reactivity and high measles specificity. The vaccine-derived Edmonston strain and measles genotype B3 were both accurately detected. Reproducibility studies demonstrated a coefficient of variation of ≤ 4.5%. Accuracy study data exhibited 100% correlation (n=160). A cross-site study testing with an external RT-PCR method demonstrated 86.2% overall agreement; of 29 negative samples, 3 resulted as positive with our assay that confirmed upon repeat testing.

Conclusion: A reverse transcription, laboratory-developed nucleic acid amplification test for detection of measles showed excellent performance in laboratory validation studies. Analytical sensitivity and specificity were acceptable for patient testing with a low LoD (6-57 copies/mL), high degree of accuracy in a blinded method comparison (100% correlation), and no observed cross-reactivity (both by in vitro and in silico analyses). This validation study supports the use of this assay for detection of measles in clinical samples.

A large-scale investigation of antibiotic resistance genes and associated environmental factors in *Listeria* isolated from natural environments across the United States.

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Listeria, including *L. monocytogenes*, the etiological agent of listeriosis in humans, can rapidly acquire antibiotic resistance genes (ARGs), posing increasing food safety and public health concerns. It is believed that environmental changes may select particular antimicrobial-resistant clones that can then widely spread in nature. There is thus an urgent need to advance our understanding of the distribution of ARGs in *Listeria* in the environment and underlying ecological mechanisms. To this end, we leveraged our nationwide genomic and environmental dataset to characterize ARGs in 596 *Listeria* isolates detected in soil samples collected across the US. We further identified environmental factors that may promote its ARG acquisition using statistical tests. We found that the *lmo0919* gene, which confers lincomycin resistance, and *sul* gene, which confers sulfonamide resistance, are the most prevalent AMR genes (present in >80% isolates), followed by *fosX*, *norB*, and *lmo1695* genes, which confer fosfomycin, quinolone, and gallidermin/α-defensin resistance, respectively. *Listeria* isolates from the eastern US harbor significantly higher diversity and richness of ARGs compared to the remaining regions. The richness and diversity of ARGs in *Listeria* is significantly associated with soil property, climate, and land-use variables and can be accurately predicted by these variables using machine learning models (area under receiver operating characteristic curve of 0.85 and 0.81, respectively). Collectively, our data suggest that the distribution of ARGs in *Listeria* in the natural environment is spatially heterogeneous, strongly shaped by environmental factors.

Lightning Talks

An unexpected role for pyrimidine biosynthesis in regulating virulence gene expression in *Staphylococcus aureus*

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Staphylococcus aureus is a commensal bacterium that colonizes the anterior nares of up to 30% of the human population. As an opportunistic pathogen, *S. aureus* is a leading cause of skin and soft tissue infections, osteomyelitis, and bloodstream infections. Staphylococcal virulence is potentiated by the production of a slew of virulence factors that facilitate nutrient acquisition and assist in evading and subverting the immune response. One key virulence regulatory system is the Sae two-component system (TCS), which upregulates the production of over 20 different virulence factors. The SaeS sensor kinase is a member of the intramembrane family of histidine kinases (IMSKs) about which we know relatively little. A hallmark of these IMSKs is the lack of a signal binding domain. Thus, how these IMSKs sense their signals and elicit changes in gene expression is unclear. To gain insight how SaeS functions mechanistically, we performed transposon mutagenesis screening for factors required for SaeS kinase activity. Herein, we report that disrupting *pyrE* (orotate phosphoribosyltransferase; *de novo* pyrimidine biosynthesis) results in a 3-fold drop in Sae-dependent promoter activity. Disrupting other steps in pyrimidine biosynthesis also lowers Sae TCS activity. Using PhosTag electrophoresis and western blotting we show pyrimidine limitation affects SaeS kinase activity. To determine the extent of pyrimidine limitation we performed RNA-Seq and identified additional Sae-dependent and Sae-independent effects on gene expression. Taken together, our data suggest that the Sae TCS integrates pyrimidine sufficiency with virulence factor production. Current work is focused on the mechanism by which pyrimidines affect SaeS kinase activity.

β-mannan metabolism in *C. japonicus* requires both a mannosyl-glucose phosphorylase and a cellobiose epimerase

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Lignocellulose is a composite of diverse polysaccharides, where β-mannans constitute up to 60% of the biomass. β-mannans are a type of hemicellulose that can have diverse configurations, which include glucomannan (β-1,4-linked glucose, and mannose residues) and galactomannan (β-1,4-linked mannose with α-1,6-branched galactose). Consequently, microbial utilization of β-mannans as a carbon source requires a cadre of enzymes for its complete reduction to substituent sugars. Bacterial metabolism of the degraded oligosaccharides has generally been shown to differ depending on the degree of polymerization of the imported substrate. In the Gram-negative bacterium *Cellvibrio japonicus*, mannose does not support growth as the sole carbon source but interestingly mannobiose can be a sole carbon source. We hypothesized that *C. japonicus* imports and subsequently deconstructs mannobiose using a small operon that encodes a mannosyl-glucose phosphorylase (*mgp130A*) and a mannobiose epimerase (*epiA*). We utilized a systems biology approach to determine the physiological relevance of these gene products for mannan metabolism, and found that.transcriptomic analyses uncovered a significant change in gene expression for both *mgp130A* and *epiA* when grown on glucomannan compared to glucose. Additionally, a mutational analysis of single and double genetic deletions confirmed *mgp130A* and *epiA* are crucial to mannan metabolism. In support of previous reports that characterized the biochemical significance of a mannosyl-glucose phosphorylases and mannobiose epimerases, we have now provided genetic and physiological characterization as to the biological importance of these genes for the metabolism of manno-oligosaccharides in bacteria.

Factors Associated with Success or Failure of Fecal Microbiota Transplant for Recurrent *Clostridioides difficile* Infection: 6-year Experience at a Multi-disciplinary Clinic

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Clostridioides difficile infection (CDI) has emerged as a prevalent intestinal infection, both in the hospital and community settings. Recurrent CDI (rCDI) negatively impacts quality of life. Fecal microbiota transplantation (FMT) is currently the most efficacious treatment for rCDI. However, host factors contributing to outcome of FMT have not been well established. To identify these factors, we conducted a retrospective chart review of patients who received FMT at University of Virginia Health between 2014 and 2020. The primary outcome was failure of first FMT, defined as rCDI within one year of FMT. 171 patients met eligibility criteria: 68.4% of patients were female and median age was 69. The median number of reported CDI was 4, with 61% of patients experiencing 4 or more infections. 20.5% of patients experienced FMT failure. Age 65 and older ($p = 0.0478$), male sex ($p = 0.04366$), and 4 or more CDIs ($p = 0.009143$) were significantly associated with FMT failure. When laboratory values prior to FMT were recorded, high TSH (>4.5 mIU/L) was associated with FMT failure ($p = 2.125*10^{-5}$). Trend toward higher failure rates was seen with high ESR, low zinc, and high hemoglobin, without statistical significance ($0.05 < p < 0.10$). In summary, older age, male sex, higher number of recurrences, and elevated TSH were associated with failure of FMT. Awareness of factors associated with FMT success can better inform care for patients undergoing FMT. The association of FMT failure and elevated TSH has not been previously seen, and needs further investigation.

Diversity in the regulatory mechanisms of bacterial ABC-dependent capsules synthesis

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Capsules are extracellular polysaccharide layers that protect the surface of many bacterial species from environmental stressors, host immune responses, and antimicrobial agents. The synthesis and transport of capsular polysaccharides (CPSs) is mediated by five general pathways as follows: Wzx/Wzy-dependent pathway (Group I); ATP-binding cassette (ABC) dependent pathway (groups II and III); synthase-dependent pathway; poly-γ-d-glutamate (PGA) Pathway; and the Group IV pathways. *E. coli (K1), Haemophilus influenzae,* and *Neisseria meningitidis (Nm)* are some medically important pathogens that use the ABC-dependent pathway for capsule synthesis. The ABC-dependent pathway comprises of two operons, the transport operon that is more conserved and the synthesis operon that is more divergent and it determines the serogroups of bacteria. Capsular synthesis is regulated in these bacteria species, but the evolutionary relationships of the regulatory mechanisms are not well understood. In *E. coli* (K1), typA, RfaH (NusG), MprA genes were shown to be required for capsule synthesis while the two-component regulator MisS/MisR was identified as a negative regulator in *Nm*. Among the *E. coli* TFs, BLAST analysis indicated that typA, RfaH (NusG) are present in the *Nm* genome while an MprA homolog was not detected. Conversely, while the *E. coli* genome encodes several two-component regulators, MisS/MisR homologs were not identified. The locally conserved blocks (LCB) associated with these genes were generated with the genome alignment program Mauve. *Nm* and *E. coli* typA genes do not occur in conserved blocks. Conversely, RfaH (NusG) is located in a LCB with several other translational proteins and some tRNAs both *Nm* and *E. coli*. This study also confirmed that the *Nm* intergenic region, which carries the capsule synthesis promoter, is functional in *E. coli*. Further sequence analysis identified putative binding sites for other transcription factors. However, it remains to be determined whether the exogenous *Nm* promoter is regulated by typA, RfaH (NusG), and MprA in *E. coli.*

The impact of intraperitoneally administered phages on host microbial community

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Antimicrobial resistance is a growing global health threat that necessitates the development of alternative treatments beyond current antimicrobials. Bacteriophage ("phage") therapy is a promising biotherapeutic that has seen renewed interest in recent years. Unlike broad-spectrum antibiotics, phage typically target only a single bacterial species or strain. Due to this specificity, there is a general assumption in the field that bacteriophage therapy will have minimal impacts on the host microbiome. However, few studies have thoroughly assessed changes in microbial community composition following phage administration. To fully evaluate the impact of this therapeutic modality, it is important to understand the potential effect of phage on non-target species and the composition of the gut microbiota. To test this, we administered bacteriophage individually or as cocktails to mice by intraperitoneal injection, then collected stool samples over time to assess the impact of the phage on the host gut microbiota. We isolated DNA from the stool samples and sequenced the V4 variable region on the conserved 16S rRNA gene to profile the diversity and composition of the gut microbiota. Evidence of significant perturbation of the microbiome will be followed with additional experimentation to elucidate interactions between phage treatment and the microbial community, whereas lack of significant disruption will be confirmed through sampling and repetition to support conclusions. This investigation in total will increase our understanding of phage therapy and interactions between phage, the host, and the host microbiota.

Helicobacter pylori genomic heterogeneity complicates the identification of mutant strain biofilm phenotypes

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With several characterized mechanisms for genetic variation, *Helicobacter pylori* accumulates mutations quickly relative to other bacterial species. This level of heterogeneity within a population can be advantageous to an organism that must colonize and persist in the harsh gastric environment for many years. However, this characteristic can also make it difficult to study *H. pylori in vitro*. Specifically, the genetic variation that may occur within a single passage of wild-type (WT) *H. pylori* can affect the outcomes of biological assays as well as affect the isogenic nature of constructed mutant and complementation strains. Downstream, this phenomenon can complicate our interpretation of identified phenotypes, including those for *in vitro* biofilm formation. After we failed to reproduce biofilm-related phenotypes with a series of 'isogenic' mutant strains, we sought to determine how the accumulation of mutations in *H. pylori* could affect the reliability of biofilm assay results. To this end, we plated multiple WT strains of *H. pylori* to obtain single colony isolates. Twelve isolates were then selected and tested in a 24-well crystal violet biofilm assay. We found that ~10% of these first-generation single colony isolates displayed significantly different levels of biomass formation as compared to the parental strain. In contrast, analysis of second-generation single colony isolates revealed that the biofilm phenotypes were consistently similar to the phenotypes found with their respective first-generation isolates. This was true for two well-studied WT strains of *H. pylori*, G27 and SS1. Whole genome sequencing of isolates revealed specific genetic alterations in several outer membrane proteins that are likely responsible for the unpredictable biofilm phenotypes. Ongoing studies seek to define the role of these secondary mutations in biofilm formation. Combined, these data suggest that *in vitro* passage of *H. pylori* selects for genetic alterations that affect biofilm formation. Thus, transcriptomic, screening, and/or mutagenesis approaches that do not include complementation may yield phenotypes that are the result of secondary mutations. Overall, these results have broad implications for the study of numerous *H. pylori* phenotypes and emphasize the necessity of sequence confirmation and/or genetic complementation in all *H. pylori* studies.